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(54) Title: CHIMERAS OF HEPATITIS C VIRUS AND BOVINE VIRAL DIARRHEA VIRUS

(57) Abstract

Disclosed is a polynucleotide comprising a chimeric viral RNA which contains: a 5' nontranslated region (5' NTR), an open reading frame (ORF) region, and a 3' nontranslated region (3' NTR) wherein at least one of said regions is chimeric. The chimeric region comprises a first nucleotide sequence from a pestivirus in operable linkage with a heterologous nucleotide sequence. The chimeric viral RNA is replication-competent. Preferably the pestivirus sequence is from a bovine viral diarrhea virus and the heterologous nucleotide sequence is from a hepatitis C virus. Also disclosed are a method for identifying compounds having antiviral activity against hepatitis C virus, a genetically-engineered chimeric RNA virus and a vaccine against bovine viral diarrhea virus.

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Chimeras of Hepatitis C Virus and Bovine Viral Diarrhea Virus

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Related Applications

This application claims priority to, and incorporates herein in its entirety, U.S. 60/082,964 filed April 24, 1998.

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Background of the Invention

(1) Field of the Invention

This invention relates generally to the development of therapies for treating hepatitis C virus (HCV) and bovine viral diarrhea virus (BVDV) and more particularly to the identification of such therapies using chimeric viruses comprising a genomic sequence  
15 derived from HCV and bovine viral diarrhea virus (BVDV).

(2) Description of the Related Art

The *Flaviviridae* is an important family of human and animal RNA viral pathogens (Rice, CM. 1996. *Flaviviridae*: The viruses and their replication. In: Fields BN, Knipe DM, Howley PM., eds. *Fields virology*. Philadelphia: Lippincott-Raven Publishers. pp. 931-960.)  
20 The three currently recognized genera of the *Flaviviridae* family exhibit distinct differences in transmission, host range, and pathogenesis. For example, members of the classical flavivirus genus, such as yellow fever virus and dengue virus, are typically transmitted to vertebrate hosts via arthropod vectors and cause acute self-limiting disease (Monath TP, Heinz FX. 1996. Flaviviruses. In: Fields BN, Knipe DM, Howley PM., eds. *Fields virology*. New York:  
25 Raven Press. pp. 961-1034). The pestiviruses, such as bovine viral diarrhea virus (BVDV) and classical swine fever virus (CSFV), cause economically important livestock disease and are spread by direct contact or the fecal-oral route (Thiel et al., 1996. Pestiviruses. In: Fields BN, Knipe DM, Howley PM., eds. *Fields virology*. New York: Raven Press. pp. 1059-1073).  
30 The most recently characterized *Flaviviridae* genus is the hepacivirus genus, the sole member of which is the common and exclusively human pathogen, hepatitis C virus (HCV). HCV is

transmitted by contaminated blood or blood products and is the most common agent of non-A, non-B hepatitis, affecting more than 1% of the population worldwide (Houghton, 1996. Hepatitis C viruses. In: Fields BN, Knipe DM, Howley PM., eds. *Fields virology*. Philadelphia: Lippincott-Raven Publishers. pp. 1035-1058.). Unlike flavivirus and pestivirus infections, which are usually eliminated by host immune response, chronic HCV infections are common and can cause mild to severe liver disease including cancer.

Despite these differences, members of the *Flaviviridae* family share common structural features and gene expression strategies. Virus particles consist of a lipid bilayer envelope with embedded transmembrane glycoproteins surrounding a protein-RNA nucleocapsid. Genome RNAs are single-stranded of positive polarity, and function as the sole mRNA species for translation of a single long open reading frame (ORF). This ORF is translated into a polyprotein which is processed by cellular and viral proteases into mature viral proteins. Structural proteins destined for incorporation into virus particles are encoded in the N-terminal portion of the polyprotein, while the nonstructural proteins which form components of the viral RNA replicase are encoded in the remainder.

Replication of the *Flaviviridae* RNA genome occurs via synthesis of a full-length negative-strand intermediate and is asymmetric, favoring synthesis of positive-strand RNAs. However, little is known about the details of this process. For all three genera of the *Flaviviridae* family, full-length functional cDNA clones have been constructed and RNAs transcribed from these cDNA templates are infectious. For flaviviruses and pestiviruses, mutagenesis of these clones and efficient RNA transfection of permissive cell cultures provides a means of probing the role of *cis* RNA elements and viral proteins in replicase assembly and function. Such analyses are not yet possible for HCV since this virus is unable to replicate efficiently in cell culture.

Like many other RNA viruses, it is believed the 5' and 3' terminal sequences of the *Flaviviridae* contain conserved *cis*-elements important for translation, RNA replication, and packaging (Bukh et al., *Proc. Natl. Acad. Sci. USA* 89:4942-4946, 1992; Deng et al., *Nucleic Acids Res.* 21:1949-1957, 1993; Cahour et al., *Viol.* 207:68-76, 1995; Kolykhalov et al., *J. Virol.* 70:3363-3371, 1996; Men et al., *J. Virol.* 70:3930-3937, 1996; Tanaka et al., *J. Virol.* 70:3307-3312, 1996; Huang HV. 1997. Evolution of the alphavirus promoter and the *cis*-acting sequences of RNA viruses. In: Saluzzo J-F, Dodet B. eds. *Factors in the emergence of arbovirus diseases*. Paris: Elsevier Press, pp. 65-79; Mandl et al., *J. Virol.* 72:2132-2140, 1998). The 5' nontranslated region (NTR) functions initially at the level of translation. Similar to most cellular mRNAs, flavivirus genome RNAs are translated in a cap-dependent manner. These RNAs contain a 5' cap structure that is presumably added by virus-encoded

RNA triphosphatases, guanylyl-, and methyl-transferases (Rice, 1996, *supra*). In contrast, the translational strategy employed by pestiviruses and HCV is more similar to that of the picornaviruses. These RNAs appear to be uncapped and contain long 5' NTRs with *cis* RNA elements that function as internal ribosome entry sites (IRES) for translation initiation at the polyprotein AUG (Lemon et al., *Semin. Virol.* 8:274-288, 1997).

The 5' NTRs of HCV and BVDV have a similar structural and functional organization despite containing only short stretches of high sequence identity (Wang et al., *Curr. Top. Microbiol Immunol.* 203:99-115, 1995; Lemon et al., 1997, *supra*). The IRES within each NTR is located at the 3' end of the NTR at a position proximal to the AUG initiation codon of the ORF. Although the 5' terminal sequence of each of these viruses is apparently not required for IRES function (Rijnbrand et al., *FEBS Lett* 365:115-119, 1995; Honda et al., *Virology.* 222:31-42, 1996; Rijnbrand et al., *J. Virol.* 71:451-457, 1997), these sequences are highly conserved among different strains of HCV (Bukh et al., *Proc. Natl. Acad. Sci. USA*:89:4942-4946, 1992) or BVDV (Deng et al., 1993, *supra*), suggesting they play other roles in viral replication. For example, sequences in the 5' NTR may be required for regulating translation versus initiation of negative-strand RNA synthesis. Such regulation could occur by direct interaction of 5' and 3' RNA elements or indirectly, via RNA-protein interactions. Sequences in the 5' NTR may also modulate packaging versus translation. Finally, sequences complementary to the 5' NTR, which are located at the 3' end of negative-strand RNA, are likely to function in the initiation of positive-strand RNA synthesis.

The HCV 3' NTR contains an internal polypyrimidine tract followed by a highly conserved sequence of 98 bases at the 3' terminus, which has been shown to be required for replication of HCV (U.S. Application Serial No. 08/811,566).

Further elucidation of the role of sequences in the HCV 5' and 3' NTRs has been hampered by the inefficient replication of HCV in cell culture. This aspect of HCV biology also makes it difficult to identify and test possible antiviral compounds for activity against HCV. Thus, a need exists for a system which facilitates investigation of HCV replication and therapeutic approaches to control HCV infections.

### Summary of the Invention

Briefly, therefore, the present invention provides novel compositions and methods for studying HCV replication which are based on the discovery that chimeras of HCV and BVDV genomic sequences can be constructed that are able to replicate in cell culture. The BVDV-specific sequence provides the chimeric viral nucleic acid with the ability to replicate in cell culture, while the HCV-specific sequence allows the chimeric viral nucleic acid to be used to

screen possible compounds for anti-viral activity against HCV. It is believed that similar replication-competent chimeras can be constructed from HCV and other pestiviruses.

Thus, in one embodiment, the present invention provides a novel, chimeric viral RNA in which at least one of the 5' NTR; ORF and 3' NTR regions is chimeric and comprises a nucleotide sequence from the corresponding region of a pestivirus in operable linkage with a nucleotide sequence from the corresponding region of an hepatitis C virus (HCV). The chimeric viral RNA is replication-competent. In preferred embodiments, the pestivirus is BVDV.

In other embodiments, the invention provides a polynucleotide comprising a DNA-dependent promoter operably linked to a cDNA of a chimeric viral RNA as described above and cells transiently transfected or stably transformed with the polynucleotide. In some embodiments the cDNA may encode a dominant selectable marker or an assayable reporter.

In yet another embodiment, the invention provides a method for identifying compounds having anti-HCV activity. The method comprises providing a first cell containing a chimeric viral nucleic acid derived from HCV and a pestivirus as described above and a second cell containing the pestivirus, and then comparing the replication efficiency of the chimeric viral nucleic acid in the presence and absence of a test compound to the replication efficiency of the pestivirus in the presence and absence of the test compound, wherein a greater reduction in compound-induced replication efficiency of the chimeric viral nucleic acid than the pestivirus indicates the compound has anti-HCV activity.

The invention also provides a genetically-engineered virus which comprises a chimeric viral nucleic acid derived from HCV and a pestivirus as described above. In one embodiment the genetically-engineered virus comprises virus particles containing at least one HCV structural protein and is useful in a vaccine against HCV. In another embodiment, the genetically-engineered virus is attenuated as compared to the pestivirus and is useful as a vaccine against the pestivirus.

In a still further embodiment, the invention provides a replication-competent BVDV vector expressing a heterologous sequence. The BVDV vector comprises the BVDV sequences encoding the BVDV replication machinery. In some embodiments, the replication-competent BVDV vector expresses an antigen and is useful as a vaccine.

#### Brief Description of the Drawings

Figure 1 is a schematic representation of the 5' NTRs of BVDV, HCV, and EMCV showing the position of the start codons of the ORF, and the boxes indicating the canonical IRES elements.

Figure 2 shows a schematic representation of BVDV and HCV chimeras, plaque phenotypes, reticulocyte translation efficiencies relative to parental BVDV, specific infectivities in MDBK cells, titers at 24 and 48 h post-transfection (or 72 h, as indicated), and an indication of whether pseudorevertants arose with results from BVDV, 5'HCV, BVDV+HCV, and BVDV+HCVdelB3 chimeras shown in Fig. 2A and results from BVDV+HCVdelB2B3, BVDV+HCVdelB1B2B3, BVDV+HCVdelB2B3H1, and BVDV+HCVdelB2B3H1H2 shown in Fig. 2B, where N.D. means not determined.

Figure 3 illustrates the *in vitro* translation efficiency of BVDV RNA or chimeras showing bar graphs of the amount of N<sup>pro</sup>, the N-terminal protein in the BVDV ORF, expressed by the various constructs.

Figure 4 illustrates a schematic representation of EMCV chimeras, plaque phenotypes, reticulocyte translation efficiencies relative to parental BVDV, specific infectivities in MDBK cells, titers at 24 and 48 h post-transfection (or 72 h, as indicated), and an indication of whether pseudorevertants arose.

Figure 5 illustrates a pseudorevertant analyses showing in (Fig. 5A) the relative positions of mutations detected within the plaque-purified variants of passaged BVDV+HCVdelB1B2B3, 5'EMCV, and 5'HCV, and in (Fig. 5B) the 5' terminal sequences of pseudorevertants of BVDV+HCVdelB1B2B3, 5'EMCV, and 5'HCV. Novel nucleotides or sequences are shown in bold upper case type. Pseudorevertants are numbered and designated by the suffix ".R". The upper case sequence in BVDV+HCVdelB1B2B3 and BVDV+HCVdelB1B2B3.R1 is a remnant of downstream BVDV 5' NTR sequences and was created during the cloning procedures.

Figure 6 illustrates the construction of derivatives of 5'HCV designed to contain 5' termini corresponding to the sequence detected within the three analyzed pseudorevertants.

Fig. 6A shows the 5' terminal sequence of the 5'HCV derivatives with the suffix (orig) designating a derivative containing the original 5' terminal sequence of the pseudorevertant; the suffix (cons) designating a derivative containing the consensus tetranucleotide sequence 5'-GUAU at the same position; and novel sequences shown in bold upper case type. Fig. 6B shows plaque phenotypes, reticulocyte translation efficiencies relative to parental BVDV, specific infectivities in MDBK cells, and titers at 24 and 48 h post-transfection are indicated.

Figure 7 illustrates a single step growth curve for various chimeric constructs showing released virus titers measured by performing plaque assays on MDBK cells transfected with various constructs.

Figure 8 illustrates replication of BVDV RNA or chimeric derivatives in transfected MDBK cells. Equal numbers of MDBK cells ( $\sim 8 \times 10^6$ ) were electroporated with 5  $\mu$ g of

each *in vitro* synthesized RNA. MDBK cells were also transfected with infectious yellow fever 17D and Sindbis RNAs to provide molecular mass markers. One fifth of the transfected cells were seeded on 35-mm dishes and incubated in D-MEM supplemented with 10% horse serum for 6 h at 37°C. The media were then replaced with 1 ml of fresh media containing 2 g/ml of actinomycin D and 40 Ci/ml of <sup>3</sup>H-uridine. Incubations were continued for 10 h at 37°C. RNAs were isolated as described in Materials and Methods, and 1/4 of the samples was denatured in glyoxal and loaded on an agarose gel. (A) Autoradiograph of the dried gel. Only the portion of the gel containing the genomic RNAs is shown. (B) Amount of radioactivity contained within the displayed fragments as determined by scintillation counting. BVDV, lane 1; 5'HCV, lane 2; BVDV+HCVdelB2B3, lane 3; BVDV+HCVdelB2B3H1, lane 4; 5'HCV.R1orig, lane 5; 5'HCV.R1cons, lane 6; 5'HCV.R3orig, lane 7; 5'HCV.R3cons, lane 8; 5'HCV.R2orig, lane 9; 5'HCV.R2cons, lane 10; yellow fever 17D, lane 11; Sindbis, lane 12; non-transfected MDBK cells, lane 13. The experiments shown is one of two repetitions which yielded similar results.

Figure 9 illustrates the genetic map of plasmid pACNR/BUD.

Figure 10 illustrates the sequence of low copy number plasmid pACNR/BVDV NADL (circular) harboring the functional cDNA of cytopathic BVDV NADL (positive sense cDNA 5' to 3'; nt 1-12578).

Figure 11 illustrates the sequence of infectious BVDV NADL (positive sense cDNA 5' to 3').

Figure 12 illustrates the sequence of infectious non-cytopathic BVDV NADL lacking cIns (positive sense cDNA 5' to 3').

Figure 13 illustrates the sequence adapted HCV 5' NTR from 5'HCV/R1.cons (positive sense cDNA 5' to 3'; only the sequence from the 5' base to the ATG initiating the polyprotein is shown).

Figure 14 illustrates the sequence of adapted HCV 5' NTR from 5'HCV/R1.orig (positive sense cDNA 5' to 3'; only the sequence from the 5' base to the ATG initiating the polyprotein is shown).

Figure 15 illustrates the sequence of adapted HCV 5' NTR from 5'HCV/R2.cons (positive sense cDNA 5' to 3'; only the sequence from the 5' base to the ATG initiating the polyprotein is shown).

Figure 16 illustrates the sequence of adapted HCV 5' NTR from 5'HCV/R2.orig (positive sense cDNA 5' to 3'; only the sequence from the 5' base to the ATG initiating the polyprotein is shown).

Figure 17 illustrates the sequence of adapted HCV 5' NTR from 5'HCV/R3.cons (positive sense cDNA 5' to 3'; only the sequence from the 5'base to the ATG initiating the polyprotein is shown).

Figure 18 illustrates the sequence of adapted HCV 5'NTR from 5'HCV/R3.orig (positive sense cDNA 5' to 3'; only the sequence from the 5' base to the ATG initiating the polyprotein is shown).

Figure 19 illustrates the sequence of prototype HCV-BVDV chimera from pNADL/5'HR3.orig/3'H3'B with the adapted HCV 5'NTR from 5'HCV/R3.orig and tandem 3' NTR elements from HCV followed by BVDV (positive sense cDNA 5' to 3') as discussed in Example 5.

Figure 20 illustrates various deletions of the poly U track in the 3'NTR HCV sequence of BVDV/HCV chimera p5H-3H33.

Figure 21 illustrates the schematic representation of functional HCV/-BVDV chimera from pCBV/p7.

Figure 22 illustrates the sequence of functional HCV-BVDV chimera from pCBV/p7 (positive sense cDNA 5' to 3').

Figure 23 illustrates the schematic representation of a HCV/BVDV chimera with selectable marker.

Figure 24 illustrates the sequence of functional HCV-BVDV chimera from pCBV/p7/IRES-pac expressing a dominant selectable marker conferring resistance to puromycin (positive sense cDNA 5' to 3').

Figure 25 illustrates the schematic representation of a bicistronic HCV/BVDV chimera.

Figure 26 illustrates the sequence of functional bicistronic chimera expressing the entire HCV structural region derived from plasmid pNADL/BI#41/HCV str (positive sense cDNA 5' to 3')

#### Description of the Preferred Embodiments

In accordance with the present invention, the inventors herein have succeeded in generating HCV-BVDV chimeric RNAs which are replication competent. Such chimeras are useful in screening compounds *in vitro* for antiviral activity against HCV. In addition, it is believed that *in vivo* replication of HCV-BVDV chimeras according to the invention may be attenuated as compared to wild-type BVDV and thus may be useful in vaccinating animals against BVDV. It is also believed that the HCV chimeric structures described herein for BVDV are applicable to other pestiviruses.

In the context of this disclosure, the following terms will be defined as follows unless otherwise indicated:

"Cis-acting sequences" means the nucleotide sequences from an RNA virus genome that are necessary for recognition of the genomic RNA by specific protein(s) of the RNA virus or host cell that carry out replication, transcription, translation or packaging of the genome.

"Genetically-engineered virus" means any virus whose genome is different than that of a wild-type virus due to a human-made deletion, insertion, or substitution of one or more nucleotides to the wild-type viral genome.

"Infectious" when used to describe a virus means the virus is capable of entering cells and initiating a virus replication cycle, whether or not this leads to the production of new RNA virus particles.

"Nucleotide sequence" as used herein refers to DNA and the corresponding RNA sequence where relevant. It will be understood that sequences shown in the Figures are DNA versions of the RNA sequence and that chimeric molecules of the invention may comprise RNA molecules or cDNA copies of such RNA molecules.

"Replication-competent" as applied to a chimeric HCV-pestivirus RNA means the RNA is capable of RNA-dependent replication in at least one cell type that supports replication of the wild-type parental pestivirus. The number of replicated RNA molecules produced by an HCV-pestivirus chimeric RNA of the invention is at least 10-fold higher than the limit of detection, which is typically 10 to 100 molecules. More preferably, chimeric RNA production by the HCV-pestivirus chimeric RNA is at least  $10^2$  to  $10^3$ -fold higher than the detection limit. The replication-competent chimeric RNA replicates at an efficiency that is preferably, at least 0.001%, more preferably, at least 0.01%, more preferably, at least 0.1%, more preferably, at least 1%, more preferably at least 10% and most preferably at least 50% up to 90% that of the parental pestivirus in the same cell type.

"Transfected cell" means a cell containing an exogenously introduced nucleic acid molecule, and includes cells that are transiently transfected with the exogenous nucleic acid.

"Transformed cell" or "stably transformed cell" means a cell containing an exogenously introduced nucleic acid molecule which is present in the cytoplasm or nucleus of the cell and may be stably integrated into the chromosomal DNA of the cell.

"Virus" means a virion, virus particle or a viral genome.

A chimeric viral RNA according to the invention is designed to comprise a 5' NTR, an ORF, and a 3' NTR, at least one of which is a chimeric region containing two operably linked nucleotide sequences that are from the same region of a pestivirus and an HCV.



Pestivirus-specific sequences useful in the invention can be taken from the appropriate genomic region of any cytopathic or noncytopathic type I or type II BVDV isolate, classical swine fever virus (CSFV) isolate, or border disease viral isolate. For a list of pestiviruses, see Thiel, H.-J., P. G. W. Plagemann, and V. Moennig. 1996. Pestiviruses, p. 1059-1073. In B. N. Fields, D. M. Knipe and P. M. Howley (ed.), Fields Virology. Raven Press, New York.

5 HCV-specific sequences can be taken from any strain or isolate of HCV, including but not limited to HCV-1, HCV-1a, HCV-1b, HCV-1c, HCV-2a, HCV-2b, HCV-2c, HCV-3a. Preferably, the parental pestivirus is a cytopathic strain of BVDV and the parental HCV strain is HCV-1.

10 The pestivirus- and HCV-specific sequences are operably linked in the chimeric region, meaning the sequences are arranged such that the resulting chimeric structure is functional in the context of replication of the pestivirus. For example, in one preferred embodiment the chimeric viral RNA comprises a chimeric 5' NTR which comprises a BVDV-specific 5' terminal sequence of 5'-(G/A)UUAU and an IRES derived from HCV, with

15 the ORF and the 3' NTR consisting of a sequence from the same regions of BVDV. The BVDV-specific sequences at the 5' terminus and in the ORF and 3' NTR are chosen such that they are functional in the context of BVDV, meaning the chimeric viral RNA expresses the replication machinery of BVDV and this replication machinery is capable of replicating the chimeric RNA. In addition, translation of the BVDV ORF in the chimeric viral RNA is

20 dependent upon a functional HCV IRES. The presence of a functional HCV IRES in this chimera allows the chimera to be used to screen for compounds that target the HCV IRES and thereby inhibit translation of the BVDV ORF as well as replication of the chimeric virus. Such compounds would be expected to also inhibit translation of the ORF in a wild-type HCV and consequently inhibit HCV replication.

25 Compounds that could be screened for anti-HCV activity using this and other HCV-BVDV 5' NTR chimeras include but are not limited to antisense RNAs, RNA decoys that bind proteins involved in recognition of the HCV-specific sequences, ribozymes, and small molecule inhibitors of critical RNA-protein interactions. The use of such substances for therapeutic applications are known in the art. See, e.g., Amarzguioui M, et al., "Hammerhead

30 ribozyme design and application." *Cell Mol Life Sci.* 1998 Nov;54(11):1175-202; Welch PJ, et al., "Expression of ribozymes in gene transfer systems to modulate target RNA levels.", *Curr Opin Biotechnol.* 1998 Oct;9(5):486-96; Bramlage B, et al. "Designing ribozymes for the inhibition of gene expression."; *Trends Biotechnol.* 1998 Oct;16(10):434-8; Gewirtz AM, et al. "Nucleic acid therapeutics: state of the art and future prospects."; *Blood.* 1998 Aug

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It is contemplated that a number of replication-competent chimeric structures can be made that allow the function of various HCV sequence elements and proteins to be studied and targeted in drug screening assays. For example, the invention includes replication-competent HCV-pestivirus chimeras having a chimeric ORF. One such chimeric ORF is one

35 comprising an HCV sequence encoding the structural proteins and a pestivirus sequence

encoding the nonstructural proteins. It is believed that upon introduction into a cell, such a HCV-BVDV ORF chimera will produce HCV-like virus particles that will be released from the cell and capable of infecting cells normally infected by wild-type HCV, i.e., cells expressing an HCV receptor such as human CD81. Such ORF chimeras would be useful to screen compounds for drugs that inhibit formation, release or entry of HCV particles. In addition, ORF chimeras that produce virus particles containing at least one HCV structural protein would be useful as vaccines against HCV. Other ORF chimeras contemplated by the invention include, for example, chimeras comprising a pestivirus sequence encoding structural proteins and an HCV sequence encoding one or more nonstructural proteins such as the NS3 protease, NS4A cofactor, NS5A phosphoprotein/interferon resistance determinant and/or the NS5B polymerase. Replication of such ORF chimeras would be dependent upon the function of the HCV nonstructural protein(s) and these ORF chimeras could be used to screen for drugs that target the HCV nonstructural protein(s) as well as to screen for and map potential drug resistance mutations in HCV nonstructural proteins. In addition, HCV-pestivirus ORF chimeras could be useful for developing alternative *in vivo* animal models for HCV replication and HCV-associated hepatocellular carcinoma to evaluate antivirals and anti-tumor agents.

The invention also provides replication-competent HCV-pestivirus chimeras having a chimeric 3' NTR which contains one or more conserved elements of the HCV 3' NTR. Such 3' NTR chimeras would be useful for screening or evaluating compounds targeted against the HCV 3' NTR. Compounds that could be screened include antisense RNA molecules, ribozymes and small molecule inhibitors of critical RNA-protein interactions. One 3' NTR chimera according to the invention comprises a BVDV 5' NTR, BVDV ORF and a chimeric 3' NTR which consists of an HCV-specific sequence derived from the HCV 3' NTR immediately followed by a BVDV 3' NTR. The HCV-specific 3' NTR that allows for replication in the context of BVDV has a deletion in the 3' NTR poly (U) tract but has all the other HCV 3' NTR elements, including the 98 bp 3' terminal conserved element.

HCV-pestivirus chimeras included within the scope of the invention include those comprising combinations of chimeric regions, i.e., 5' NTR and ORF chimeras; 5' NTR and 3' NTR chimeras; ORF and 3' NTR chimeras; and chimeric RNAs in which each of the 5' NTR, ORF and 3' NTR regions comprise an HCV sequence operably linked to a pestivirus sequence.

The invention also provides chimeric RNAs having two ORFs, or bicistronic HCV-pestivirus chimeras. Bicistronic chimeras contemplated by the invention include structures in which the first ORF contains one or more HCV genes and is followed by a second IRES

operably linked to a second ORF encoding the pestivirus replicase machinery. It is also contemplated the first ORF may encode a heterologous sequence such as an antigen.

It is believed that many HCV-pestivirus chimeras of the invention will be attenuated as compared to the parental wild-type pestivirus. Such attenuated chimeric RNA genomes would be candidate vaccines in the form of live-attenuated virus particles or as RNA or cDNA "genetic" vaccines.

The invention also includes vaccines against HCV which comprise an immunogenically-effective amount of HCV-pestivirus particles or nucleic acid. Anti-HCV vaccines comprising virus particles should preferably contain one or more HCV structural proteins.

The therapeutic or pharmaceutical compositions of the present invention can be administered by any suitable route known in the art including for example by injection such as intraperitoneal, intravenous, subcutaneous, intramuscular, transdermal, intrathecal or intracerebral injection. Administration can be either rapid as by injection or over a period of time as by slow infusion or administration of slow release formulation.

Compositions according to the invention can be employed in the form of pharmaceutical or veterinary preparations. Such preparations are made in a manner well known in the pharmaceutical and veterinary arts. One preferred preparation utilizes a vehicle of physiological saline solution, but it is contemplated that other pharmaceutically acceptable carriers such as physiological concentrations of other non-toxic salts, five percent aqueous glucose solution, sterile water or the like may also be used. It may also be desirable that a suitable buffer be present in the composition. Such solutions can, if desired, be lyophilized and stored in a sterile ampoule ready for reconstitution by the addition of sterile water for ready injection. The primary solvent can be aqueous or alternatively non-aqueous.

The carrier can also contain other pharmaceutically-acceptable excipients for modifying or maintaining the pH, osmolarity, viscosity, clarity, color, sterility, stability, rate of dissolution, or odor of the formulation. Similarly, the carrier may contain still other pharmaceutically-acceptable excipients for modifying or maintaining release or absorption or penetration across the blood-brain barrier. Such excipients are those substances usually and customarily employed to formulate dosages for parenteral administration in either unit dosage or multi-dose form or for direct infusion into the cerebrospinal fluid by continuous or periodic infusion.

It is also contemplated that certain formulations containing a chimeric virus according to the invention are to be administered orally. Such formulations are preferably encapsulated and formulated with suitable carriers in solid dosage forms. Some examples of suitable

carriers, excipients, and diluents include lactose, dextrose, sucrose, sorbitol, mannitol, starches, gum acacia, calcium phosphate, alginates, calcium silicate, microcrystalline cellulose, polyvinylpyrrolidone, cellulose, gelatin, syrup, methyl cellulose, methyl- and propylhydroxybenzoates, talc, magnesium, stearate, water, mineral oil, and the like. The formulations can additionally include lubricating agents, wetting agents, emulsifying and suspending agents, preserving agents, sweetening agents or flavoring agents. The compositions may be formulated so as to provide rapid, sustained, or delayed release of the active ingredients after administration to the patient by employing procedures well known in the art. The formulations can also contain substances that diminish proteolytic degradation and promote absorption such as, for example, surface active agents.

The specific dose is calculated according to the approximate body weight or body surface area of the patient or the volume of body space to be occupied. The dose will also be calculated dependent upon the particular route of administration selected. Such calculations can be made without undue experimentation by one skilled in the art. Exact dosages are determined in conjunction with standard dose-response studies. It will be understood that the amount of the composition actually administered will be determined by a practitioner, in the light of the relevant circumstances including the condition or conditions to be treated, the choice of composition to be administered, the age, weight, and response of the individual patient, the severity of the patient's symptoms, and the chosen route of administration. Dose administration can be repeated depending upon the pharmacokinetic parameters of the dosage formulation and the route of administration used.

Replication-competent HCV-pestiviruses are generated by choosing the HCV function or sequence element desired to be studied. The HCV sequence can be obtained from a plasmid clone of a partial or full HCV genome using PCR to amplify a target region containing the desired sequence or by restriction enzyme digestion. The HCV fragment is then inserted into the desired location of a clone of the pestivirus genome using standard techniques. Desired portions of the pestivirus genome may be deleted before or after addition of the HCV fragment. The recombinant genome is then transfected into a cell that supports replication of the parental pestivirus genome and their ability to replicate using standard assays. For example, replication can be assessed by virus-induced cytopathic effect; plaque formation; detection of viral antigens and/or viral RNA accumulation; and by plaque assay measuring released infectious virus. The inventors herein have found that the BVDV RNA replication machinery works in many cell types, including bovine, hamster, mouse and human cells. It has also been reported that BVDV RNAs can amplify in other cell types including human hepatoma lines and hepatocytes (Behrens SE, et al., *J Virol.* 1998 Mar;72(3):2364-72).

The host cell range for a particular chimera will be dependent upon the properties of that chimera as empirically determined.

As described below, some chimeras do not replicate stably as indicated by heterogeneity in the size of plaques produced by the chimeric virus. Upon passage, pseudorevertants can frequently be isolated that are capable of stable replication. Such pseudorevertants will have one or more deletions or base substitutions in the HCV and/or pestivirus sequences. Information derived from these gain-of-function mutations can be used to define the elements necessary for generating stable, replication-competent chimeras of HCV and a pestivirus.

The invention provides a method for screening compounds for antiviral activity against HCV. The method involves comparing a test compound's effect on replication of a chimeric HCV-pestivirus RNA molecule as described above with the compound's effect on replication of the parental pestivirus. Compounds which have a greater effect on replication of the chimeric virus than the pestivirus are likely directed against the HCV portion of the chimera. Typically, the method is performed by providing duplicate cell cultures containing a chimeric viral RNA which is replication-competent in that cell, treating one of the culture with the test compound, and then measuring the replication efficiency of the chimeric RNA in both cultures. Any effect induced by the compound is compared against the compound's effect on replication of the parental pestivirus in cells of the same type. This control assay is preferably performed at the same time using the same culture conditions.

The cells used in the screening assay can be prepared by transiently transfecting the cells with the desired chimeric RNA molecule as described below. Alternatively, it is contemplated that the chimeric RNA molecule can be constitutively expressed in the cell by transfecting the cell with a polynucleotide comprising a cDNA of the chimeric RNA operably linked to a DNA-dependent promoter. The chimeric cDNA may include a selectable marker which would allow for selection of cells expressing the chimeric RNA. It is also envisioned the selectable marker could be a dominant marker that allows selection of cells expressing chimeras having adaptive mutations or selection of cells permissive for virus replication (Frolov et al., *J. Virol.* 73:3854-3865, 1999). It is also contemplated the cDNA could express a reporter gene that could be assayed to measure RNA replication.

Alternatively, chimeric virus particles are incubated with a cell permissive for infection by the pestivirus in the presence or absence of the test compound and then replication of the chimeric virus is measured and compared to the replication of the parental pestivirus incubated with the same cell type in the presence or absence of the test compound.

Inhibition of replication can be measured in many ways, including assaying for the reduction of virus-induced cytopathic effect; inhibition of plaque formation, reduced production of viral antigens as detected by immunofluorescence assay; reduced viral RNA accumulation; reduction in released infectious virus from treated and untreated control and chimera samples using a plaque assay. In addition, it is contemplated that a cell line that is designed for pestivirus-specific transactivation of a reporter gene could be used directly or in lieu of a plaque assay. The reporter gene is operably linked to a promoter that is activated upon infection by the chimeric virus and production of the viral transactivator protein.

Preferred embodiments of the invention are described in the following examples.

Other embodiments within the scope of the claims herein will be apparent to one skilled in the art from consideration of the specification or practice of the invention as disclosed herein. It is intended that the specification, together with the examples, be considered exemplary only, with the scope and spirit of the invention being indicated by the claims which follow the examples.

#### Example 1

This example illustrates the construction and analysis of 5' HCV-BVDV chimeras as reported in detail in Frolov et al. (*RNA* 4:1418-1435, 1998) which is incorporated in its entirety by reference. A functional clone of BVDV (Mendez et al., *J. Virol.* 72:4737-4745, 1998) was used to construct and characterize a series of 5' NTR chimeras with sequences derived from HCV and the picornavirus, encephalomyocarditis virus (EMCV). The results help to define the requirements of a functional BVDV 5' NTR and provide replication-competent BVDV-HCV chimeras dependent on a functional HCV IRES.

#### Example 2

This example illustrates the construction of chimeras for expressing additional functional portions of the HCV genome by addition of further HCV sequence downstream from the functional or adapted HCV 5'NTR chimeras fused in-frame to the BVDV ORF.

One such construct (Figure 21) involves fusion of HCV sequences to BVDV sequences in the p7 protein coding region (at a convenient BseRI restriction site). Both HCV and BVDV encode a p7 protein that is located immediately downstream of the E2 protein.

The p7 protein is a small hydrophobic protein of unknown function. pCBV/p7 consists of the first 79 bases of the BVDV 5'NTR encoding stem loop structure B1' and B1, followed by the entire HCV 5'NTR, the entire HCV structural protein coding region and the first 36 amino acids of HCV p7 fused to the C-terminal 31 amino acids of BVDV p7. The fused p7 gene is followed by the remainder of the BVDV ORF including the entire nonstructural region and the BVDV 3' NTR. Transfection of MDBK cells with the RNA corresponding to this

sequence (Figure 22) leads to replication of the chimeric RNA and production of the expected HCV and BVDV polyprotein cleavage products. Variations on this strategy are envisioned in which all or part of the HCV polyprotein and cis elements important for RNA packaging can be expressed in viable chimeras. In addition the BVDV replicase regions for either cytopathic or non-cytopathic pestiviruses (like NADL cIns-) can be used. Transfection of cells permissive for HCV particle, assembly, release and reinfection with this chimeric RNA can be used to make HCV-like particles. These particles and this infection system can be used (i) to screen for specific inhibitors of HCV particle, assembly, release and reinfection, (ii) for identifying antibodies capable of neutralizing HCV infectivity and (iii) as live or inactivated vaccines. Furthermore, this embodiment of the invention demonstrates that the BVDV RNA replication machinery can be used for expression of heterologous RNA and polypeptide sequences and can be used as a vehicle for RNA or DNA "genetic" vaccination in which the BVDV replicase amplifies the level of antigen expression by cytoplasmic RNA-dependent replication.

### Example 3

This example illustrates chimeric RNA's that are modified to express dominant selectable markers, assayable markers or FACS sortable markers.

Such variants can be used to select for chimeras capable of replication in particular cell types, or to screen for cell types that are permissive for replication of the chimeric RNA. Selectable markers include, but are not limited to, the genes encoding puromycin resistance (puromycin N-acetyl transferase; PAC), neomycin resistance, blasticidin resistance, hygromycin resistance, etc. Assayable markers include, but are not limited to, the genes encoding B-galactosidase, luciferase, B-glucuronidase, etc. Easily sortable molecules include single chain antibodies, cell surface markers, and non-toxic protein markers like green fluorescent protein. In a specific example (Figures 23 and 24), the RNA encoded by pCBV/p7 was modified to include a cassette at the beginning of the BVDV 3'NTR that is comprised of the EMCV IRES driving the gene encoding PAC. This chimeric RNA can replicate, expresses PAC and confers resistance to puromycin resistance. This property can be used to select for variants of the chimera that are capable of noncytopathic replication in desired cells type and also provides a means of showing that cells harbor a functional chimeric RNA. Desired variants can be identified, cloned and further characterized as described in Example 1. Of note, is that this location in the BVDV genome and this strategy for expressing heterologous genes may also be applied to using infectious attenuated



pestiviruses as gene expression vectors and as chimeric live vaccines against other animal pathogens.

#### Example 4

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This example illustrates the use of the bicistronic strategy as an alternative to the in-frame fusions described in Example 2.

A specific example is shown in Figure 25 and its sequence as Figure 26. In this bicistronic chimera, the 5' sequences are identical to that of pCBV/p7 except that the HCV ORF continues to include the first 246 amino acids of NS4B. The HCV sequence is followed by the EMCV IRES fused to BVDV Npro, the N-terminal 10 aa of BVDV C, the C-terminal 19 aa of C, 9 N-terminal amino acids of Erns, 48 C-terminal amino acids of E2 and the remainder of the BVDV NADL ORF and 3' NTR. The constructed BVDV ORF encodes a functional BVDV RNA replicase. The deletions in the N-terminal portion of this ORF were designed to preserve proper membrane topology and processing of the replicase. The bicistronic chimeric RNA can replicate upon transfection of permissive BVDV host cells.

#### Example 5

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This example illustrates 3'NTR chimeras. Although initial attempts to recover viable chimeric viruses in which the BVDV 3'NTR was completely replaced by that of HCV were unsuccessful, a strategy similar to that detailed in Example 1 has produced chimeras that harbor the conserved elements of the HCV 3'NTR. An initial tandem 3'NTR construct was made in which the HCV 3'NTR was engineered to follow the BVDV ORF. The complete BVDV 3'NTR was position 3' to the HCV 3' NTR after a short heterologous sequence. This sequence of this parental construct, which replicated poorly, is shown in Figure 19 RNAs transcribed from this plasmid were of low specific infectivity suggesting that revertants or pseudorevertants might have arisen. Indeed isolation and sequence analysis of several independent plaque-forming variants revealed that deletions in the HCV poly U tract of various lengths had occurred. These revertant sequences are shown in Figure 20. When these altered HCV 3'NTRs were reconstituted into the original tandem 3' NTR parent, they gave rise to plaque forming RNA transcripts of high specific infectivity, demonstrating that these alterations restored the ability of the chimeric RNA to replicate. Large deletions in the U tract gave rise to virus with more robust replication and larger plaques while stably maintaining the conserved HCV 3'NTR 98-base element and the polypyrimidine "transition" region. Such

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chimeric viruses can now be used to screen and evaluate antisense, ribozyme, and other therapeutics targeted against this conserved HCV RNA element that is essential for replication.

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## Materials and Methods

### Plasmid Constructs

pACNR/BVDV NADL was previously described (Mendez et al., 1998, *supra*). pBVDV is a derivative of pACNR/BVDV NADL which contains a G→T transversion at nt 14994 that creates an *Xba* I site upstream of the T7 promoter (T. Myers & C.M. Rice, 10 unpubl.). To facilitate construction of the chimeras, subclones were created. First, two fragments were isolated by PCR amplification of p90/HCVFLIongpU (Kolykhalov et al., *Science* 277:570-574, 1997) with primers #498 (5'-TGTACATGGCACGTGCCAGCCCC) and #498 (5'-GATCAACTCCATGGTGCACGGTCT) and pBVDV with primers #481 (5'-AGACCGTGCACCATGGAGTTGATC) and #482 (5'-15 CGTTTCACACATGGATCCCTCCTC). These two fragments were digested with *Apa* I and ligated to produce a fragment containing a fusion of the HCV 5' NTR to the BVDV ORF. This fragment was digested with *Sac* I and ligated into pGEM3Zf(-) which had been digested with *Sma* I and *Sac* I to produce the subclone pGEM498-*Sac* I. Next, a fragment containing the BVDV 5' NTR was synthesized by PCR amplification of pBVDV with primers #183 (5'-20 TTTTCTAGATAATACGACTCACTATAGTATACGAGAATTAGAAAAGGCACTCG) and #480 (5'-GGGGGCTGGCACGTGCCATGTACA). This fragment was digested with *Xba* I and *Bsr* G I and ligated into pGEM498-*Sac* I digested with the same two enzymes, to create the plasmid pGEMXbal-*Sac* I. pGemXbal-*Sac* I contains a tandem fusion of the BVDV 5' NTR, the HCV 5' NTR, and the 5' portion of the BVDV N<sup>pro</sup> gene. pBVDV + HCV was 25 created by digesting pGEMXbal-*Sac* I with *Xba* I and *Sac* I and ligating the fragment into pBVDV digested with the same two enzymes, and as such pBVDV + HCV contains the T7 promoter, followed by the entire 385-nt 5' NTR of BVDV, a GT dinucleotide (nt 386-387), the entire 341-nt 5' NTR of HCV (nt 388-728), and the sequence of the BVDV NADL strain including the ORF and 3' NTR. Derivatives of pBVDV + HCV containing deletions within 30 the BVDV 5' NTR and/or the HCV 5' NTR were created in the subclone pGEMXbal-*Sac* I, as described below, prior to ligation into *Sba* I- and *Sac* I-digested pBVDV. For making deletions, restriction sites with non-compatible protruding ends were treated with the Klenow fragment of DNA polymerase I prior to ligation. For creation of pBVDV + HCVdelB3 (deletion of nt 174-374, inclusive), pGEMXbal-*Sac* I was digested with *Afl* II and 35 *Bsr* G I. For pBVDV + HCVdelB2B3 (deletion of nt 67-374), pGEMXbal-*Sac* I was digested

with *Avr* II and *Bsr*G I. For pBVDV + HCVdelB1B2B3 (deletion of nt 33-374), pGEMXbal-SacI was digested with *Sna*B I and *Bsr*G I. For pBVDV + HCVdelB2B3H1 (deletion of nt 67-3396), pGEMXbal-SacI was digested with *Avr* II and *Xcm* I. For pBVDV + HCVdelB2B3H1H2 (deletion of nt 67-513), pGEMXbal-SacI was digested with *Avr* II and *Bsg* I. For pBVDV + HCVdelB2B3H3 (deletion of nt 67-374, 518-704), subclone pGEMXbal-SacI delB2B3 was digested with *Sma* I. p5'HCV was created by digesting p90/HCVlionsgpU with *Xba* I and *Nru* I and ligating the fragment into pBVDV + HCV digested with the same two enzymes.

The EMCV plasmid, pEC<sub>g</sub>, was provided by Ann Palmenberg and is described elsewhere (Hahn et al., *J. Virol* 69:2697-2699, 1995). p5'EMCV contains the entire 710 nt of the 5' NTR of EMCV, followed by the open reading frame of BVDV and the 3' NTR. One extra G residue was added between the T7 promoter and the first nucleotide of the EMCV 5' NTR to facilitate efficient in vitro transcription. Convenient restriction sites within the BVDV 5' NTR or the EMCV 5' NTR were used to create additional chimeras. Sites with noncompatible protruding ends were treated with the Klenow fragment of DNA polymerase I prior to ligation. For example, the plasmid pBVDV + EMCVdelA contains nt 1-378 of BVDV 5' NTR fused with nt 45-710 of EMCV (the *Bsr*G I site of BVDV ligated to the *Eco*R V site of EMCV), pBVDV + EMCVdelB3A contains nt 1-173 of BVDV fused with nt 45-710 of EMCV (the *Afl* II site of BVDV ligated to the *Eco*R V site of EMCV). pBVDV + EMCVdelB2B3A contains nt 1-66 of BVDV fused with nt 45-710 of EMCV (the *Avr* II site of BVDV ligated to the *Eco*R V site of EMCV). pBVDV + EMCVdelB3ABC contains nt 1-173 of BVDV fused with nt 161-710 of EMCV (the *Afl* II site of BVDV ligated to the *Psp*1405 site of EMCV). pBVDV + EMCVdelB2B3ABC contains nt 1-66 of BVDV fused with nt 161-710 of EMCV (the *Avr* II site of BVDV ligated to the *Psp*1406 site of EMCV). pBVDV + EMCVdelB3A-H contains nt 1-101 of BVDV fused with nt 289-710 of EMCV (the *Nhe* I site of BVDV ligated to the *Avr* II site of EMCV). pBVDV + EMCVdelB2B3A-H contains nt 1-62 of BVDV fused with nt 289-710 of EMCV (the *Avr* II site of BVDV ligated to the *Avr* II site of EMCV). The schematics of the chimeric 5' NTRs are presented in Figures 2 and 4.

All other heterologous 5' NTRs used in the study were generated by PCR using an oligonucleotide complementary to nt256-272 of the HCV 5' NTR and primers containing the sequence of the *Xba* I restriction site followed by the T7 promoter, the heterologous sequences found in sequenced pseudorevertants, or sequences corresponding to different regions of the HCV 5' NTR. All the fragments were subcloned into the plasmid, pRS2 (a derivative of pUC19), sequenced, and recloned into the p5'HCV plasmid by replacing the

fragment between the *Xba* I site located upstream of the T7 promoter and the *Nhe* I site (nt 249-254) in the 5' NTR of HCV.

#### Cell cultures

5 MDBK cells were obtained from M. Collett (ViroPharma, Inc.) and BT cells were obtained from the American Type Culture Collection (Rockville, Maryland). Cells were grown in Dulbecco's modified Eagle medium (D-MEM) supplemented with 10% horse serum and sodium pyruvate.

#### Transcriptions and transfections

10 All the designed plasmids, including pBVDV and the chimeric derivatives, were digested to completion with *Sda* I (*Sse*83871), purified by phenol extraction, precipitated by ethanol, and dissolved in water. The transcription reactions were performed in the T7 Megascript kit (AMBION) using the conditions recommended by the manufacturer. Reactions were incubated at 37°C for 1 h, and <sup>3</sup>H-UTP was added to the reaction to quantify the RNA synthesis. The quality of the synthesized RNAs was checked by agarose gel  
15 electrophoresis, and samples containing 50-60% of full-length RNA were used for electroporations and in vitro translations. The reaction mixtures were aliquoted and stored at -70°C prior to electroporation or in vitro translations.

Transfection was performed by electroporation of MDBK cells using previously described conditions (Mendez et al., 1998, *supra*). Two micrograms of in vitro synthesized  
20 RNA, corresponding to approximately 1 µg of the full-length transcript, were used per electroporation. In standard experiments, ten-fold dilutions of electroporated cells were seeded in 6-well tissue culture plates containing 5 x 10<sup>5</sup> naive MDBK cells per well. After 1 h of incubation at 37°C in a 5% CO<sub>2</sub> incubator, cells were overlaid with 3 ml of 0.6% LE Sea Kem agarose (FMC Bioproducts) containing minimal essential medium supplemented  
25 with 5% horse serum. Plaques were stained with crystal violet after 3 days incubation at 37°C. The rest of the transfected cells was seeded into 100-mm dishes and incubated for approximately 48 h or until cytopathic effect was observed in virtually all cells. Samples of the media were taken at 24 and 48 h, and virus titers were determined as described above and previously (Mendez et al., 1998, *supra*).

#### 30 Analysis of the 5' ends of viral genomes

Sequencing of the 5' ends of selected variants of BVDV was performed on plaque-purified viruses. Plaques were typically isolated from the agarose overlay without staining with neutral red. Virus was eluted in 1 ml of D-MEM/10% horse serum for several hours and was used to infect 5 x 10<sup>5</sup> MDBK cells in 35-mm dishes. After 1 h of virus adsorption of 37

°C, an additional 1 ml of D-MEM/10% horse serum was added to the dishes, and incubation was continued for 36-48 h until cytopathic effect was observed in virtually all cells.

Fifty microliters of harvested viral stocks were clarified by low speed centrifugation, and viral RNAs were isolated by TRIzol reagent (Gibco-BRL) using the protocol recommended by the manufacturer. Sequencing of the 5' termini was performed using an oligonucleotide/cDNA-ligation strategy described elsewhere (Troutt et al., *Proc. Natl. Acad. Sci. USA* 89:9823-9825, 1992). The primer S1 (5'-GTCGTTTCACACATGGATCC), complementary to nt 710-729 of the BVDV genome, was used for cDNA synthesis. A phosphorylated oligonucleotide tag (5'-GACTGTTGTGGCCTGCAGGGCCGAATT) with an amino group on the 3' terminus was ligated to the first strand cDNA (Troutt et al., 1992, *supra*). One tenth of this reaction mixture was used for PCR amplification. The primers for PCR amplification were as follows: primer A (5'-GCCCTGCAGGCCACAACAGTC), complementary to the tag; primer B (5'-TCAGGCAGTACCACAA) complementary to nt 281-296 of the HCV 5' NTR; and primer C (5'-GGAATGCTCGTCAAGAAGACAG), complementary to nt 268-289 of the EMCV 5' NTR. The primer pairs of A + B or A + C were used for analysis of the pseudorevertants of 5'HCV and BVDV + HCVdelB1B2B3 or 5'EMCV, respectively. For the 5'HCV pseudorevertants, one tenth of the ligation mixture was used for an additional PCR reaction. This fragment was synthesized using primer S1, describe above, and a primer corresponding to nt 147-175 of the HCV genome. Fragments were purified by agarose gel electrophoresis and cloned into the plasmid pRS2. Multiple independent clones were sequenced by the standard dideoxy-mediated chain termination methods using the Sequenase version 2.0 DNA Sequencing Kit (USB).

#### Cell-free translation

Cell-free translation reactions were performed in reticulocyte extracts (Promega) using conditions recommended by the manufacture. Usually 0.1-1 µg of the same in vitro synthesized RNAs used in transfection experiments were used in 25 µl translation reactions. After 45 min of incubation at 30 °C, 2 µl were dissolved in 10 µl of sample buffer, and those samples were analyzed by sodium dodecyl sulfate PAGE. Labeled proteins were visualized by autoradiography of the dried gel. The efficiency of translation was measured using phosphorimager analysis (Molecular Dynamics) by comparing the radioactivity in the band corresponding to the N<sup>pro</sup> protein. In preliminary experiments, an eightfold increase in incorporation was observed for translation of 4 µg versus 0.4 µg BVDV transcript RNA. Quantitative data were obtained from reactions using subsaturating (0.4 µg) amounts of BVDV or BVDV chimera transcript RNAs.

### Analysis of virus specific RNAs

The protocols used for radioactive labeling of virus-specific RNAs are described in the appropriate figure legends. RNAs were isolated from the cells by using TRIzol reagent as recommended by the manufacturer (Gibco-BRL). After denaturation with glyoxal in dimethylsulfoxide, cellular RNAs were analyzed by electrophoresis in a 1% agarose gel containing a 10 mM phosphate buffer. Pieces of the dried gel containing the appropriate RNA bands were excised, and their radioactivity measured by liquid scintillation counting.

### **Results**

#### **Features of the BVDV, HCV, and EMCV 5' NTRs important for chimera design**

Schematic representations of the proposed secondary structures of the 5' NTRs of HCV, BVDV, and EMCV are shown, and the location of each IRES is indicated in Figure 1. EMCV is a member of the cardiovirus genus within the family *Picornaviridae*. While not a member of the *Flaviviridae*, EMCV is similar to HCV and BVDV in that it is a positive-strand RNA virus shown to contain an IRES within its 5' NTR (Jang et al., *J. virol* 62:2636-2643, 1988). Based on their proposed secondary structures, the HCV IRES and the BVDV IRES have been classified as type 3 IRESs, while the EMCV IRES is classified as a type 2 IRES (Lemon & Honda, *Siemin. Virol.* 8:274-288, 1997). However, these three IRESs as well as IRESs from other members of the *Flaviviridae* and the *Picornaviridae* have been proposed to contain a common structural core (Le et al., *Virus Genes* 12:135-147, 1996).

The model for the secondary structure of the 341-nt HCV 5' NTR has been refined by enzymatic and chemical analysis of synthetic transcripts (Brown et al., *Nucl. Acids. Res.* 20:5041-5045, 1992; Wang et al., *J. Virol* 68:7301-7307, 1994; Honda et al., *RNA* 2:955-968, 1996; Lima et al., 1997). This element contains four discrete hairpins (referred to here as H1, H2, H3 and H4) and a pseudoknot at the base of hairpin H3 (Wang et al., 1995). The secondary structure of the 385-nt BVDV 5' NTR has not been as extensively studied, but is proposed to be similar to that of HCV (Brown et al., 1992) with four discrete hairpins (referred to here as B1', B1, B2, and B3) and a pseudoknot at the base of B3 (Rijnbrand et al., 1997). The secondary structure of the longer (>700 nt) EMCV 5' NTR consists of a series of hairpins A-M (Duke et al., 1992; Hoffman & Palmenberg, 1996). Recently, a revised model of the EMCV 5' NTR suggests moderately different secondary structures for the C and G subregions, and significantly different secondary structures for the I-M subregion (Palmenberg & Sgro, 1997).

For HCV, H1 is nonessential for IRES function (Reynolds et al., 1995; Rijnbrand et al., 1995; Honda et al., 1996b; Reynolds et al., 1996; Kamoshita et al., 1997) and its deletion

has actually increased translation efficiency in some analyses (Rijnbrand et al., 1995; Honda et al., 1996b). Most studies have found that hairpin H2 and H3 and the pseudoknot are essential for IRES function (Wang et al., 1993; Rijnbrand et al., 1995; Honda et al., 1996b). However, two studies indicate that H2 may not be essential (Tsukiyama-Kohara et al., 1992; Urabe et al., 1997). The 3' boundary of the HCV IRES is more controversial. The IRES clearly extends to the AUG initiation codon. However, some studies indicate that sequences affecting the efficiency of translation initiation extend into the ORF (Reynolds et al., 1995; Honda et al., 1996a; Honda et al., 1996b; Lu & Wimmer, 1996). By analogy to the HCV IRES and the related pestivirus CSFV IRES, the BVDV IRES probably requires hairpins B2 and B3 and the pseudoknot for function, with B1' and B1 probably not required for IRES activity (Poole et al., 1995; Rijnbrand et al., 1997). For EMCV, hairpins H-L have been shown to be required for IRES function in mono- or dicistronic constructs (Jang & Wimmer, 1990; Duke et al., 1992). The remaining portion of the EMCV 5' NTR is thought to be required for RNA replication or unknown steps in viral replication that are important for pathogenesis (Duke et al., 1990; Martin & Palmenberg, 1996).

**Replacement of the BVDV 5' NTR with the HCV 5' NTR results in a large decrease in specific infectivity**

Since the BVDV 5' NTR and the HCV 5' NTR are proposed to have similar RNA secondary structure and functional organization, an experiment was performed to test whether the BVDV 5' NTR could be replaced by the HCV 5' NTR. p5' HCV has an exact replacement of the BVDV 5' NTR with that of HCV (Fig. 2A) while the coding sequence and 3' NTR of p5'HCV are identical to pBVDV. Positioning of the HCV 5' NTR in such a manner was necessary since translation initiation from the HCV IRES begins at or near the AUG start codon (Honda et al., 1996a; Reynolds et al., 1995; Reynolds et al., 1996; Rijnbrand et al., 1996). The specific infectivity of 5'HCV RNA synthesized in vitro was compared to that of BVDV RNA by transfection of MDBK (bovine kidney) cells (Fig. 2A). The specific infectivity of BVDV RNA was approximately  $4 \times 10^6$  plaque forming units (PFU)/ $\mu$ g RNA. In contrast, the specific infectivity of 5' HCV RNA was near the limit of detection (30-50 PFU/ $\mu$ g RNA) and considerable plaque heterogeneity was apparent. These results suggested that the HCV 5' NTR replacement chimera might be incapable of efficient replication and plaque formation and that the plaque forming virus observed had arisen by secondary mutation(s). Sequence analysis of plaque-purified 5' HCV viruses presented below confirmed that the replicating pool of virus contained such pseudorevertants.

Next, the *in vitro* translation efficiency of these two RNAs in rabbit reticulocyte extracts was analyzed to test whether the defect in specific infectivity of 5' HCV RNA could be attributed to lower translation efficiency. Although the specific infectivity of 5' HCV RNA was reduced ~5 logs compared to BVDV RNA, its translation efficiency was only slightly reduced, ~twofold (Fig. 3, lane 1 vs. lane 2). The apparent size of the N-terminal cleavage product, N<sup>pro</sup>, was identical for both RNAs, suggesting that translation initiated with the correct AUG. These data are consistent with the hypothesis that the BVDV 5' NTR contains signals that are required for a step in replication other than translation which are not present in the 5' HCV chimera.

Given the low specific infectivity of 5' HCV RNA, an experiment was performed to test the effect of placing the BVDV 5' NTR sequence upstream of the HCV 5' NTR, resulting in tandem BVDV and HCV 5' NTRs (called BVDV + HCV). This arrangement actually decreased translation efficiency (Fig. 3, lane 14 vs. lane 1) yet restored infectivity (Fig. 2A). The plaques produced by BVDV + HCV were also heterogeneous in size, indicating that this virus was unstable. Upon passage, RT-PCR analysis indicated that pseudorevertants had indeed arisen in which portions of the BVDV and/or HCV 5' NTRs had been deleted (data not shown). These data show that sequences in the BVDV 5' NTR required for virus replication can function when placed upstream of a functional HCV IRES driving translation of the BVDV polyprotein.

#### **Hairpins B1' and B1 in conjunction with the HCV IRES are sufficient for stable and efficient BVDV replication**

The sequences within the BVDV 5' NTR that restored replication in the context of the HCV 5' NTR were mapped using three deletion variants. The deletion BVDV + HCVdelB3 removed a large portion of hairpin B3; the deletion within BVDV + HCVdelB2B3 removed hairpins B2 and B3, and the deletion within BVDV + HCVdelB1B2B3 removed hairpins B1, B2 and B3. The specific infectivities of RNAs from these deletion mutants were near that of BVDV RNA (Fig. 2). Upon passage of these viruses, RT-PCR analyses and sequencing indicated that BVDV + HCV delB3 and BVDV + HCVdelB2B3 were stably propagated and produced homogeneous plaques slightly smaller than those of wild-type BVDV (data not shown). In contrast, BVDV + HCVdelB1B2B3 produced smaller heterogeneous plaques. Reverse transcription-polymerase chain reaction (RT-PCR) analysis and sequencing indicated that BVDV + HCVdelB1B2B3 underwent a reversion event described in more detail below. The translation efficiencies of these three RNAs (Fig. 3, lanes 9, 10, and 12) were similar to BVDV + HCV RNA (Fig. 3, lane 14), indicating that the deleted portions (hairpins B1, B2,



and B3) are not required for translation in the BVDV + HCV chimera. These results show that B1' and B1 are the minimal elements sufficient for stable replication in conjunction with the HCV 5' NTR.

Having shown that B1' and B1 are sufficient for replication in conjunction with the HCV 5' NTR, we next conducted a deletion analysis to determine the sequences within the HCV 5' NTR of BVDV + HCV delB2B3 required for replication. A large portion of H1 was deleted in BVDV + HCV delB2B3H1, while both H1 and H2 were deleted in BVDV + HCV delB2B3H1H2. Of these two RNAs, only BVDV + HCV delB2B3H1 was as infectious as parental BVDV RNA (Fig. 2B). However, the BVDV + HCV delB2B3H1 virus produced smaller plaques than BVDV + HCV delB2B3, indicating that hairpin H1 may augment replication of the chimera. In contrast, BVDV + HCV delB2B3H1H2 RNA was not infectious (Fig. 2B) and was translated poorly (Fig. 3, lane 11). Diminished HCV IRES activity might be due to deletion of hairpin H2 or juxtaposition of BVDV hairpins B1' and B1 with H3. A third derivative of BVDV + HCV delB2B3, with a *Sma* I-*Sma* I deletion abrogating HCV IRES function by removing H3, was also not infectious (data not shown). Thus, a 5' NTR consisting of B1' and B1 and a functional HCV IRES is sufficient for stable BVDV replication in MDBK cells. Similar results were obtained in BT cells, another BVDV-permissive continuous bovine cell line (data not shown).

## 20 Replacement of the BVDV 5' NTR with the EMCV 5' NTR

The following experiment was performed to determine whether the BVDV 5' NTR could be replaced by the 5' NTR of a more phylogenetically distant virus, EMCV. A derivative of BVDV was created, called 5' EMCV, that contains an exact replacement of the BVDV 5' NTR with the EMCV 5' NTR plus an additional guanosine residue at the 5' terminus for more efficient transcription initiation of T7 polymerase (Fig. 4A). The specific infectivity of 5' EMCV RNA was more than three orders of magnitude lower than BVDV RNA, indicating that it was defective for replication, although its specific infectivity was higher than that of 5' HCV RNA (compare Figs. 4A and 2A). Similar to 5' HCV, 5' EMCV produced heterogeneous plaques, and sequence analysis indicated that pseudorevertants had arisen. The lower specific infectivity of 5' EMCV RNA was not likely because of a defect in translation, since the translation efficiency of 5' EMCV RNA was about threefold higher in vitro than that of BVDV RNA (Fig. 3, lane 20 vs. lane 19).

Similar to BVDV + HCV, it was also determined whether the BVDV 5' NTR at the 5' end of the 5' EMCV RNA would increase its specific infectivity. BVDV + EMCVdelA (Fig. 4A) contained the entire BVDV 5' NTR in tandem with the EMCV 5' NTR lacking a portion

of hairpin A. BVDV + EMCVdelA RNA had a specific infectivity near that of BDVD RNA (compare Figs. 4A and 2A) despite having a lower translation efficiency than 5' EMCV (Fig. 3, lane 21 vs. lane 20). - Similar to the results with BVDV + HCV, this implicates the added BVDV 5' NTR sequence for a step in viral replication other than translation. Two derivatives of BVDV + EMCVdelA that contain deletions of portions of the BDVD 5' NTR but maintain the sequence of B1' and B1, BDVD + EMCVdelB3A and BVDV + EMCVdelB2B3A (Fig. 4A), also were infectious. These derivatives had translation efficiencies near that of the parental BVDV + EMCVdelA (Fig. 3, compare lanes 15 and 16 with lane 21). This demonstrated that hairpins B1' and B1 were sufficient for replication in conjunction with a large portion of the EMCV 5' NTR. Derivatives of BVDV + EMCVdelB3A or BVDV + EMCVdelB2B3A that contain further deletions of EMCV (BVDV \_ EMCVdelB3ABC and BVDV + EMCVdelB2B3ABC in particular) were translated efficiently (Fig. 3, lanes 17 and 18) and were infectious (Fig. 4B). This indicates that the chimeras did not require putative EMCV RNA replication signals (Martin & Palmenberg, 1996). However, derivatives with deletions extending into the canonical EMCV IRES were not infectious. For example, BVDV + EMCVdelB3A-H and BVDV + EMCVdelB2B3A-H, in which a portion of hairpin H is deleted, were not infectious (Fig. 4B) and were inefficiently translated in vitro (Fig. 3, lanes 22 and 23). It should be noted that all of the BVDV + EMCV chimeras produced plaques of heterogeneous size, indicating some instability.

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#### **Relatively simple 5' NTR mutations are observed in adapted pseudorevertants**

As mentioned previously, BVDV + HCVdelB1B2B3 did not replicate stably as indicated by the heterogeneity in the size of plaques produced by this virus. Upon passage and selection of medium plaque-producing variants, 5' RACE analysis and sequencing indicated that nt 1-26 had been deleted in the pseudorevertants, removing a large portion of B1' which was apparently deleterious in the absence of B1. This deletion results in the 5' terminal sequence 5'GUAUCG which is identical to the first six bases of BVDV genome RNA (Fig. 5) and is repeated at positions 27-32.

Analysis of the passaged 5' EMCV virus indicated that the replicating progeny had also undergone a simple deletion of sequence at the 5' end to generate more efficiently replicating variants (Fig. 5). After electroporation, the 5' EMCV virus pool was passaged 5 times at a multiplicity of infection of 0.1-1 PFU/cell on MDBK or BT cells, and the 5' termini of three randomly picked plaques were sequenced. For all three plaques selected, nt 2-209 had been deleted, again creating a genome RNA with the 5' terminal tetranucleotide sequence 5'-GUAU.

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Analysis of the 5' HCV progeny indicated that more complicated variants had arisen. Most small plaque-producing variants were unstable and quickly reverted to medium plaque-producing variants. However, one small plaque-producing variant and two stable medium plaque-producing variants were isolated. 5' terminal sequences of the variants were amplified by rapid amplification of cDNA ends (RACE) and cloned into a plasmid vector, and sequences for several independent colonies were determined. The sequence of three clones of the small plaque-producing virus (5'HCV.R1) contained a deletion of HCV sequence from nt 1-34 and an addition of the dinucleotides 5'-AU in two clones and 5'-GU in the third clone. This creates a 5' terminus of 5'-(G/A) UAA (Fig. 5B), reminiscent of the first three bases of the BVDV genome RNA (5'-GUA). Both medium plaque variants appeared to have arisen by RNA recombination with non-viral sequences (Fig. 5). One medium plaque variant (5' HCV.R2) had deleted the first 21 bases of the HCV sequence and contained instead a heterologous sequence of 22 bases. BLAST searches revealed a perfect match between this sequence and a sequence in a human retina cDNA of unknown function (Tsp509I). The second medium plaque variant (5' HCV.R3) had also undergone a possible recombination event leading to the addition of 12 nt to the 5' end of the HCV sequence. Given its short length, multiple matches were found in the database with this sequence. As for the small plaque variant, sequencing of multiple clones revealed heterogeneity at the extreme 5' end, with either G or A identified as the 5' base. Remarkably, for both medium plaque variants, the fused heterologous sequence began with the tetranucleotide sequence 5'-(G/A) UAU (Fig. 5B). For all three variants, sequencing of the entire 5' NTR and a portion of the N<sup>pro</sup> coding region revealed only these changes at the 5' termini.

#### **5' NTR sequence changes are sufficient for the pseudorevertant phenotypes**

To assess the importance of these alterations at the 5' terminus of the 5' HCV pseudorevertants, derivatives of 5' HCV were created with the changes determined by 5' RACE (Fig. 6A) and analyzed the specific infectivities of these RNAs (Fig. 6B). Corresponding to the small plaque variant, a derivative called 5' HCV.R1 orig was engineered which contained a 5' NTR consisting of the dinucleotide 5' -GU at the 5' terminus of HCV nt 35-341. This results in a 5' terminus consisting of 5'-GUAA. 5'HCV.R1 orig RNA had a specific infectivity at least four orders of magnitude higher than 5' HCV RNA (Figs. 6B and 2A). This demonstrates that this 5' NTR structure is sufficient for phenotypic reversion to high specific infectivity. However, small plaques and considerable heterogeneity were observed for 5'HCV.R1 orig suggesting that additional mutations may be present in the original small plaque variant.

The engineered derivative 5'HCV.R2orig had a 5' NTR consisting of 22 nt of Tsp509I-homologous sequence followed by HCV nt 22-341. Another construct, called 5'HCV.R3orig was made, which has the 12 nt of the other heterologous sequence fused to the intact HCV 5' NTR. Specific infectivities for both these derivatives were essentially the same as observed for wild type BVDV RNA ( $2-4 \times 10^6$  PFU/ $\mu$ g; Fig. 6B). Transfection with these transcripts produced medium plaques, as observed for the original variants, and this phenotype was stable upon passaging. These results show that the altered 5'NTR sequences were responsible for the pseudorevertant phenotypes rather than changes elsewhere in their genomes.

#### **Addition of the tetranucleotide sequence 5'-GUAU to the HCV 5' NTR allows efficient BVDV replication**

For all three 5' HCV variants studied, as well as the BVDV + HCV delB1B2B3 and 5'EMCV pseudorevertants, 5' NTR alterations seemed to involve creation of a three- or four-base "consensus" sequence identical to the 5' terminus of BVDV genome RNA. To test the importance of this sequence, as opposed to fused heterologous sequences, we created a set of variants with the BVDV 5' tetranucleotide sequence linked to the HCV 5' NTR or the deletion/recombinant break points identified during sequence analysis of the 5' HCV pseudorevertants (Fig. 6A). 5' HCV.R1cons had the tetranucleotide sequence 5'-GUAU fused to HCV nt 35-341. 5'HCV.R2cons had the 5'-GUAU tetranucleotide sequence fused to HCV nt 22-341. 5'HCV.R3cons contained the tetranucleotide sequence 5'-Guau fused to the intact 5' terminus of the HCV NTR. RNAs from all three of these derivatives had specific infectivities more than five orders of magnitude higher than 5'HCV and comparable to parental BVDV (Fig. 6B).

There were, however, significant differences between the phenotypes of some of these derivatives versus the reconstructed pseudorevertants. As mentioned above, 5'HCV.R1orig yielded tiny and small plaques and produced low virus yields even after 48 h. In contrast, the addition of four bases rather than two bases (5'-GUAU vs. 5'-GU) yielded virus with near wild-type plaque morphology (Fig. 6B) and growth Rates (Fig. 7). In the case of the smaller deletion, 5'HCV.R2orig and 5'HCV.R2cons were indistinguishable, suggesting that, other than the 5' four bases, the fused heterologous sequences were dispensable. This was not the case, however, for the chimera containing the 5'-GUAU tetranucleotide sequence

fused to the intact HCV 5' NTR. 5'HCV.R3cons produced small plaques (Fig. 6B) and grew more slowly than 5'HCV.R3orig (Fig. 7) suggesting that the sequence/structure of the sequences downstream of the 5' four bases can affect replication efficiency.

**5 The tetranucleotide sequence 5'-GUAU is important for efficient BVDV RNA accumulation**

Next, the effects of the different 5' termini on virus-specific RNA accumulation directly after transfection were analyzed. This allowed a direct comparison between 5'HCV and the reconstructed pseudorevertants as well as selected BVDV + HCV deletion constructs.

10 MDBK cells were transfected with in vitro synthesized RNAs and labeled for 10 h beginning at 5 h post-transfection with <sup>3</sup>H-UTP in the presence of actinomycin D (Fig. 8). RNA replication of the 5' HCV chimera was severely impaired to a level below detection (Fig. 8, lane 2). In contrast, every 5' NTR alteration of 5' HCV that increased RNA specific infectivity and allowed efficient virus growth led to readily detectable viral RNA

15 accumulation. Addition of B1' and B1 to the 5' terminus of the HCV 5' NTR restored RNA replication to a level ~50% of that observed for BVDV (BVDV + HCVdelB2B3; Fig. 8, lane 3 vs. lane 1). BVDV + HCVdelB2B3H1 displayed reduced RNA synthesis compared to BVDV + HCVdelB2B3 (Fig. 8, lane 4 vs. lane 3) perhaps explaining its small plaque phenotype and suggesting a possible positive role for H1 in replication of this chimera.

20 5'HCV.R1orig, which had exhibited plaque heterogeneity and slow growth, accumulated less RNA when compared to 5'HCV.R1cons (Fig. 8, lane 5 vs. lane 6). 5'HCV.R2orig and 5'HCV.R2cons showed similar RNA accumulation (Fig. 8, lane 9 vs. lane 10) consistent with their medium plaque phenotypes; and 5'HCV.R3cons exhibited reduced RNA synthesis compared to 5'HCV.R3orig (Fig. 8, lane 8 vs. lane 7), consistent with their small-versus

25 medium-plaque phenotypes.

Although these RNA phenotypes are complex, the most striking result is that addition of the B1' B1 hairpins, addition of heterologous 5' sequences terminating with 5'-GUAU or simply fusion of this tetranucleotide sequence with the HCV 5' NTR or short 5' truncations of the HCV 5' NTR all dramatically upregulated RNA accumulation. This occurred without

30 increasing translation efficiency, at least as measured in a cell-free assay (Fig. 3, compare lanes 3-8 to lane 1), suggesting that these sequences function at the level of RNA replication or stability.

### Discussion

The work presented here helps to define the requirements for a functional BVDV 5' NTR. The BVDV-specific 5' NTR sequences required for efficient replication in cell culture are minimal and consist of the 5' terminal sequence, 5'-GUAU. The sequence 5'-AUAU, detected for some pseudorevertants, may also be functional but this was not tested for technical reasons. This simple 5'-terminal tetranucleotide sequence, which is conserved among pestiviruses (Ruggli et al., 1996; Becher et al., 1998), was shown to function in the context of functional IRES elements derived from the hepatitis C virus HCV or the picornavirus EMCV. As discussed below, this may indicate that the 5' signals required for BVDV RNA replication are rather simple or that elements in these heterologous IRESs can functionally replace deleted BVDV sequences.

Sequences at the extreme 5' end of BVDV genome RNA could modulate the efficiency of RNA accumulation by affecting RNA stability, translation, promoter efficiency, or some combination of these processes. At this time, we can not distinguish among these possibilities but favor an effect on RNA replication. The complement of the BVDV 5' sequence at the 3' end of the negative-strand RNA presumably functions in the initiation of positive-strand RNA synthesis. Thus, AUAC-3' at the 3' terminus of negative-strand RNA may be important for positive-strand RNA synthesis. Interestingly, for some positive-strand RNA viruses such as rubella virus (Pugachev & Frey, 1998), flock house virus (Ball, 1994) and turnip crinkle virus (Guan et al., 1997), only minimal *cis*-acting sequences at the 3' termini of negative-strand RNAs are required for positive-strand RNA synthesis. In contrast to the 5' NTR replacements, we were unable to generate replication-competent BVDV-HCV replacing that of BVDV (data not shown). This may indicate that the signals within the pestivirus 3' NTR required for initiation of negative-strand RNA synthesis are more complex and virus specific. Once the replication complex has assembled at the 3' NTR and transversed the RNA during negative-strand synthesis, the requirements of the 5' NTR for initiation of positive-strand synthesis may be minimal.

Although the RNA replication signals within the 5' NTR appear to be rather simple, it is possible that the signals important for RNA replication actually extend into the IRES and are more complicated. For instance, the 5'HCV pseudorevertants were more stable and grew to higher titers than the 5'EMCV counterparts, despite the fact that the 5'EMCV RNAs were translated more efficiently *in vitro*. This may indicate that the BVDV and HCV IRESs contain signals important for RNA synthesis that are absent in the EMCV IRES.

It is perhaps not surprising that 5' HCV appeared to recombine with cellular mRNAs to acquire a 5' terminus with the 5' -(G/A) UAU consensus, given that non-cytopathic strains

of BVDV can recombine with BVDV RNA or cellular mRNAs to generate cytopathic strains of BVDV (Meyers & Thiel, 1996). Presumably, this recombination event involves template switching during negative-strand RNA synthesis, as observed for polio-virus (Kirkegaard & Baltimore, 1986). In contrast to 5' HCV, simple deletions of 5' terminal viral sequences could account for the BVDV + HCVdelB1B2B3 and 5'EMCV pseudorevertants since the tetranucleotide sequence is present in these 5' NTRs upstream of functional IRES elements. Such deletions could occur by partial degradation of positive-strand template prior to negative-strand synthesis, by premature termination during negative-strand RNA synthesis, or by degradation of 3' terminal negative-strand sequence after synthesis. It is proposed that 5'HCV was forced to recombine with cellular sequences because HCV does not have an 5'-(G/A)UAU sequence upstream of its IRES. The first occurrence of an (G/A)UAUA tetranucleotide sequence is at nt 94-97 within hairpin H2, and a 5' deletion extending into this sequence would presumably inactivate or severely impair HCV IRES activity. It is interesting that BVDV + HCVdelB1B2B3 and 5'EMCV pseudorevertants were generated at much higher frequency than 5'HCV pseudorevertants. This may indicate that recombination between BVDV and cellular RNAs is a rare event compared to the processes which lead to deletion of terminal viral sequences.

Poliovirus chimeras dependent upon a functional HCV IRES have been reported (Lu & Wimmer, 1996). Interestingly, viable poliovirus chimeras were produced only when HCV sequences included both the IRES and the N-terminal portion of the HCV ORF. Nucleotide sequences or structures in the downstream ORF can modulate HCV IRES translational efficiency (see Reynolds et al., 1995; Honda et al., 1996a) but it was also suggested that the N-terminal portion of the HCV core polypeptide might be involved. In the case of our 5' HCV pseudorevertants, there is no requirement for HCV C protein sequences. Although the translation efficiency of the HCV IRES in the presence of additional HCV sequences 3' to the AUG start was not directly assessed, the HCV chimeras and pseudorevertants were translationally active and infectious in the absence of any portion of the HCV ORF. This indicates that either the HCV IRES does not extend into the HCV ORF or that the BVDV ORF contains analogous sequence which functions in our 5'HCV chimeras. There is some limited identity between HCV and BVDV within this region. For example, HCV nt 359-394 and BVDV nt 405-440 are identical at 21 of 36 positions, although identity within this sequence may be attributed to a high adenosine content. It is interesting to note that the luciferase (LUC) and chloramphenicol acetyl transferase (CAT) reporter genes previously used to detect HCV IRES activity (Tsukiyama-Kohara et al., 1992; Wang et al., 1993) also have adenosine- or purine-rich regions in relatively the same position as the HCV ORF and

BVDV ORF. It this region is indeed important for IRES activity, this may explain why some have observed that the HCV IRES does not require a portion of the HCV ORF for translation of CAT or LUC (Tsukiyama-Kohara et al., 1992; Wang et al., 1993). Point mutations and insertions within this region of HCV have been shown to reduce HCV IRES activity in vitro  
5 (Honda et al., 1996a,b).

Despite the fact that B1' and B1 are conserved among different strains of BVDV and similar hairpins are present in border disease virus and CSFV (Deng & Brock, 1993; Becher et al., 1998), B1' and B1 were dispensable for BVDV replication, provided that the 5' tetranucleotide sequence 5'-(G/A)UAU remained. This may indicate a role for B1' and B1 in  
10 viral replication in vivo that we do not observe in cell culture. It will be interesting to test the phenotype of chimeras that lack B1' and B1 in vivo to determine if they are attenuated and might serve as useful BVDV vaccines. In this vein, several studies with flaviviruses have demonstrated that alterations in 5' NTR or 3' NTR elements can lead to attenuation in vivo (Cahour et al., 1995; Men et al., 1996; Mandl et al., 1998). BVDV chimeras that utilize the  
15 HCV or EMCV IRES may also prove to be attenuated simply due to the presence of the heterologous IRES. For poliovirus, it has been shown that differences in IRES efficiency in different host-cell environments can modulate host range and virulence (Shiroki et al., 1997).

BVDV-HCV chimeras that are dependent on a functional HCV IRES may have another practical application. It may be possible to use these chimeras to screen for anti-HCV  
20 therapeutics that target the HCV IRES. Other researchers have shown antisense oligonucleotide-mediated inhibition of HCV gene expression in hepatocytes by targeting the oligonucleotides to the HCV IRES (Hanecak et al., 1996). It will be of interest to measure the efficacy of antisense oligonucleotides or ribozymes (Lieber et al., 1996) against replicating virus, and these chimeras are more useful than HCV for this purpose since they are able to  
25 replicate efficiently in cell culture. BVDV is believed to be a reasonable model of HCV replication not only because of homology and conserved motifs within the 5' NTR but also because of similarities in overall genetic organization (Rice, 1996) and polypeptide processing strategy (Tautz et al., 1997; Xu et al., 1997).

In view of the above, it will be seen that the several advantages of the invention are  
30 achieved and other advantageous results attained.

As various changes could be made in the above methods and compositions without departing from the scope of the invention, it is intended that all matter contained in the above description and shown in the accompanying drawings shall be interpreted as illustrative and not in a limiting sense.



All references cited in this specification, including patents and patent applications, are hereby incorporated by reference. The discussion of references herein is intended merely to summarize the assertions made by their authors and no admission is made that any reference constitutes prior art. Applicants reserve the right to challenge the accuracy and pertinency of

5 the cited references.

What is Claimed is:

1. A polynucleotide comprising a chimeric viral RNA which comprises:
  - (a) a 5' nontranslated region (5' NTR);
  - (b) an open reading frame (ORF) region; and
  - 5 (c) a 3' nontranslated region (3' NTR);wherein at least one of said regions is chimeric and comprises a first nucleotide sequence from a pestivirus in operable linkage with a first nucleotide sequence from an hepatitis C virus (HCV), and wherein said chimeric viral RNA is replication-competent.
- 10 2. The polynucleotide of claim 1, wherein the chimeric region is the 5' NTR and the first pestivirus nucleotide sequence is from a bovine viral diarrhea virus (BVDV).
3. The polynucleotide of claim 2, wherein the BVDV nucleotide sequence is located at the 5' terminus of the chimeric 5' NTR and comprises 5' RUAU.
- 15 4. The polynucleotide of claim 3, wherein the first HCV nucleotide sequence in the chimeric 5' NTR comprises an internal ribosome entry site (IRES).
5. The polynucleotide of claim 4, wherein the ORF and the 3' NTR consist of
- 20 second and third BVDV sequences.
6. The polynucleotide of claim 5, wherein the 5' terminal sequence comprises 5' GUAU.
- 25 7. The polynucleotide of claim 4, wherein the ORF comprises a second HCV sequence encoding at least one structural protein operably linked to a second BVDV sequence.
8. The polynucleotide of claim 1, wherein the pestivirus is BVDV and the
- 30 chimeric region is the 3' NTR.
9. The polynucleotide of claim 8, wherein the first HCV sequence in the chimeric 3' NTR comprises the HCV 98 bp 3' terminal element (SEQ ID NO:X) operably linked to the first BVDV sequence.

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10. A method for identifying compounds having antiviral activity against hepatitis C virus (HCV) comprising the steps of:

(a) providing a first cell containing a chimeric viral RNA which is replication-competent in the cell, the chimeric viral nucleic acid comprising a 5' nontranslated region (5' NTR), an open reading frame (ORF) region; and a 3' nontranslated region (3' NTR);  
wherein at least one of said regions is chimeric and comprises a first nucleotide sequence from a pestivirus in operable linkage with a first nucleotide sequence from an hepatitis C virus (HCV);

(b) providing a second cell containing the pestivirus; and

(c) comparing the replication efficiency of the chimeric viral RNA acid in the presence and absence of a test compound to the replication efficiency of the pestivirus in the presence and absence of the test compound,  
wherein a greater reduction in compound-induced replication efficiency of the chimeric viral RNA than the pestivirus indicates the compound has anti-HCV activity.

11. The method of claim 10, wherein the chimeric region is the 5' NTR and the first pestivirus nucleotide sequence is from a bovine viral diarrhea virus (BVDV).

12. The method of claim 11, wherein the BVDV nucleotide sequence is located at the 5' terminus of the chimeric 5' NTR and comprises 5' RUAU.

13. The method of claim 12, wherein the first HCV nucleotide sequence in the chimeric 5' NTR comprises an internal ribosome entry site (IRES).

14. The method of claim 13, wherein the ORF and the 3' NTR comprise second and third sequences from the BVDV.

15. The method of claim 10, wherein the pestivirus is BVDV and the chimeric region is the 3' NTR.

16. A genetically-engineered virus comprising a chimeric RNA genome which comprises:

(a) a 5' nontranslated region (5' NTR);

(b) an open reading frame (ORF) region; and

(c) a 3' nontranslated region (3' NTR);

wherein at least one of said regions is chimeric and comprises a first nucleotide sequence from a pestivirus in operable linkage with a first nucleotide sequence from an hepatitis C virus (HCV); and wherein said chimeric RNA genome is replication-competent.

5           17.     The genetically-engineered virus of claim 16, wherein the chimeric region is the 5' NTR and the first pestivirus nucleotide sequence is from a bovine viral diarrhea virus (BVDV).

10           18.     The genetically-engineered virus of claim 16, wherein the BVDV nucleotide sequence is located at the 5' terminus of the chimeric 5' NTR and comprises 5' RUAAU and the first HCV nucleotide sequence in the chimeric 5' NTR comprises an internal ribosome entry site (IRES).

15           19.     A vaccine against bovine viral diarrhea virus (BVDV) comprising an immunogenically-effective amount of a genetically-engineered virus comprising a chimeric RNA genome having:

- (a) a 5' nontranslated region (5' NTR);
- (b) an open reading frame (ORF) region; and
- (c) a 3' nontranslated region (3' NTR);

20           wherein at least one of said regions is chimeric and comprises a first nucleotide sequence from BVDV in operable linkage with a first nucleotide sequence from an hepatitis C virus (HCV), and wherein the genetically-engineered virus is attenuated as compared to BVDV.

25           20.     The vaccine of claim 19, wherein the chimeric region is the 5' NTR and the BVDV nucleotide sequence is located at the 5' terminus of the chimeric 5' NTR and comprises 5' RUAAU and the first HCV nucleotide sequence in the chimeric 5' NTR comprises an internal ribosome entry site (IRES).

30           21.     A polynucleotide comprising a chimeric viral RNA which comprises:

- (a) a 5' nontranslated region (5' NTR);
- (b) an open reading frame (ORF) region; and
- (c) a 3' nontranslated region (3' NTR);

35           wherein at least one of said regions is chimeric and comprises a first nucleotide sequence from a pestivirus in operable linkage with a heterologous nucleotide sequence and wherein said chimeric viral RNA is replication-competent.

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EMCV

HCV

BVDV

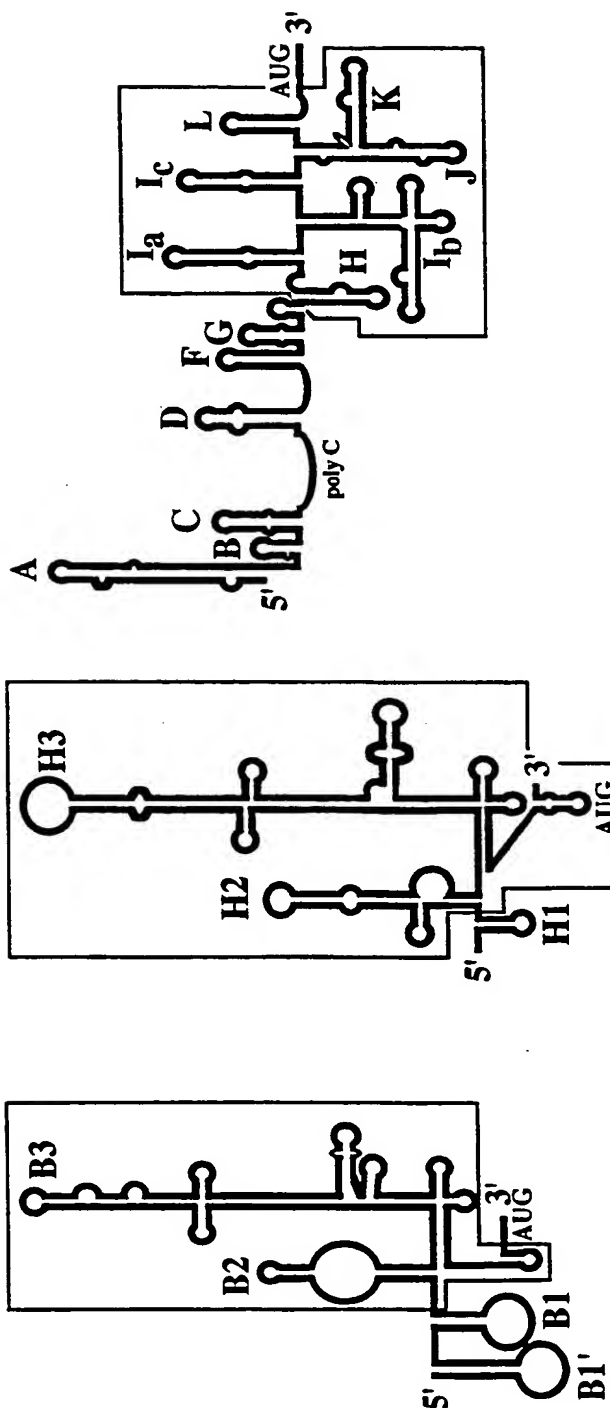
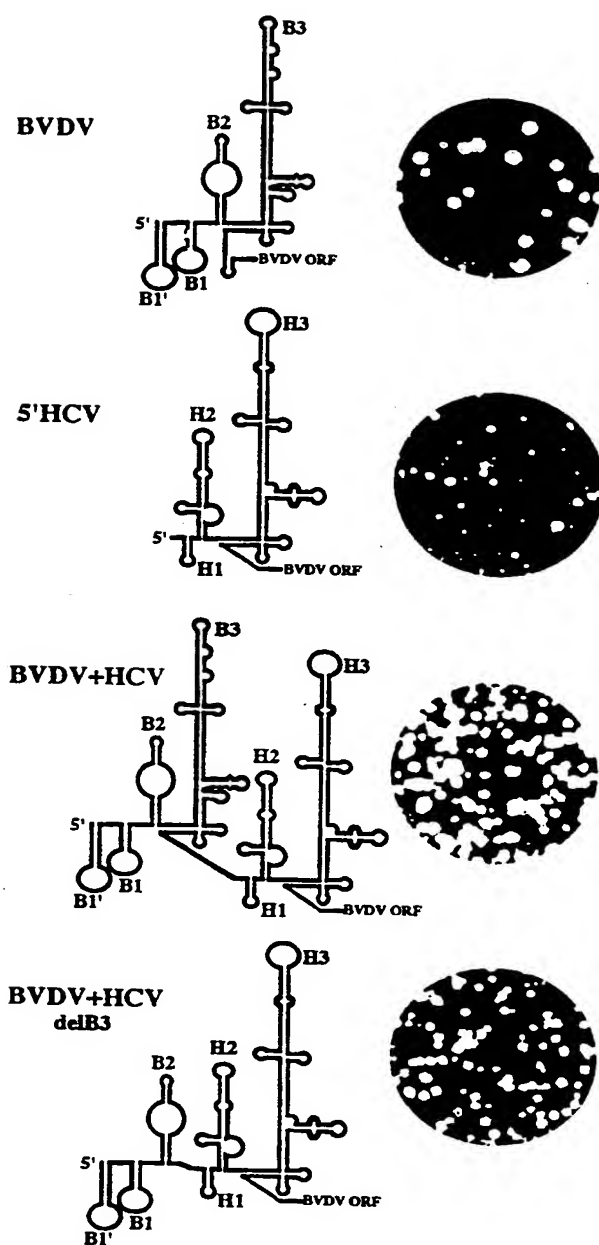


FIGURE 1

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Translation efficiency (% of BVDV)	Infectivity (PFU/ $\mu$ g of RNA)	Virus titer (PFU/ml)		Pseudorevertants
		24 h	48 h	
100	$4 \times 10^6$	$1 \times 10^8$	$8 \times 10^7$	No
50	30-50	<50	$4 \times 10^5$ (72 h)	Yes
25	$5 \times 10^6$	$1 \times 10^8$	$1 \times 10^8$	Yes
16	$4 \times 10^6$	$7 \times 10^7$	$1 \times 10^8$	N.D.

FIGURE 2A

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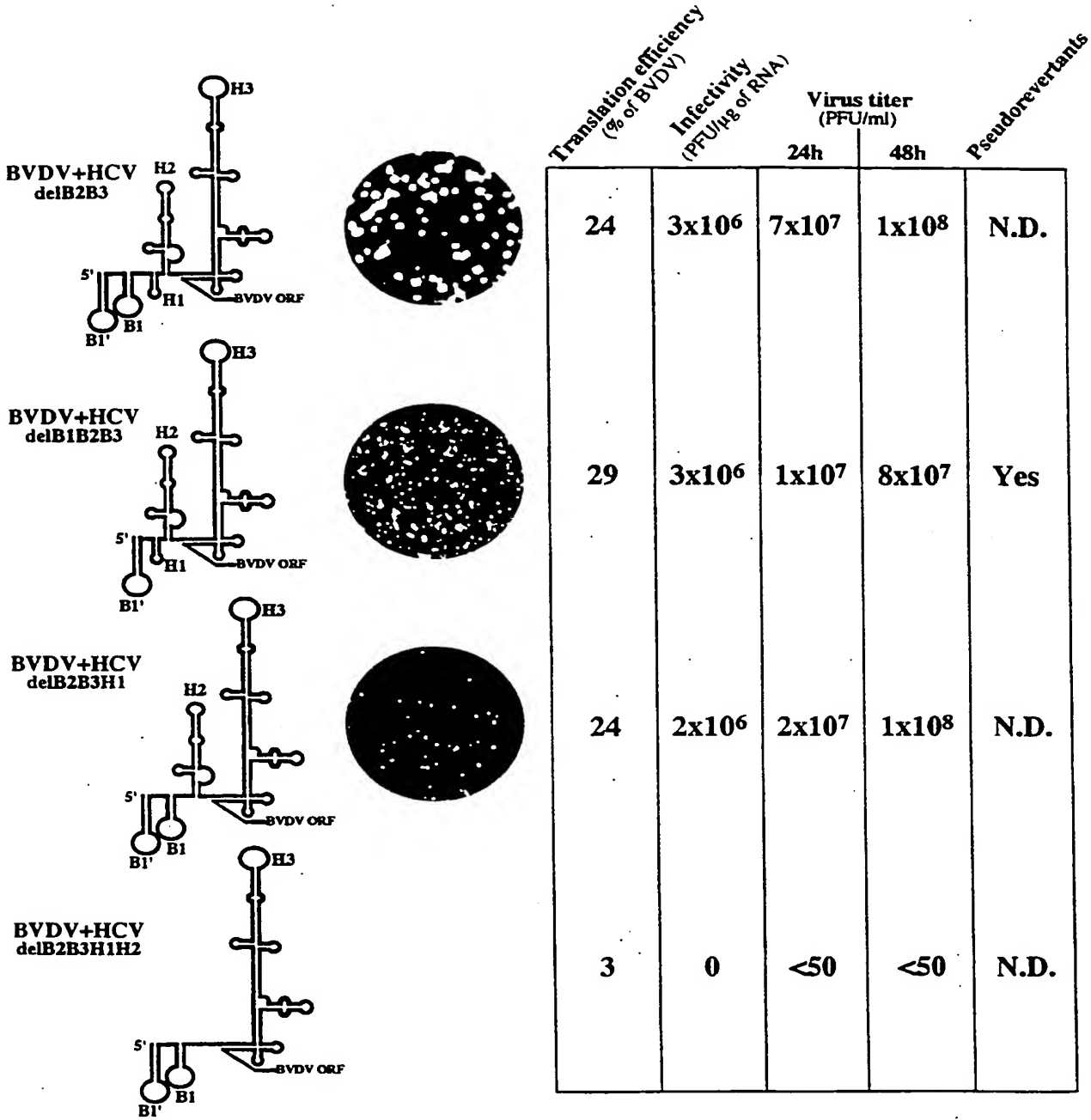


FIGURE 2B

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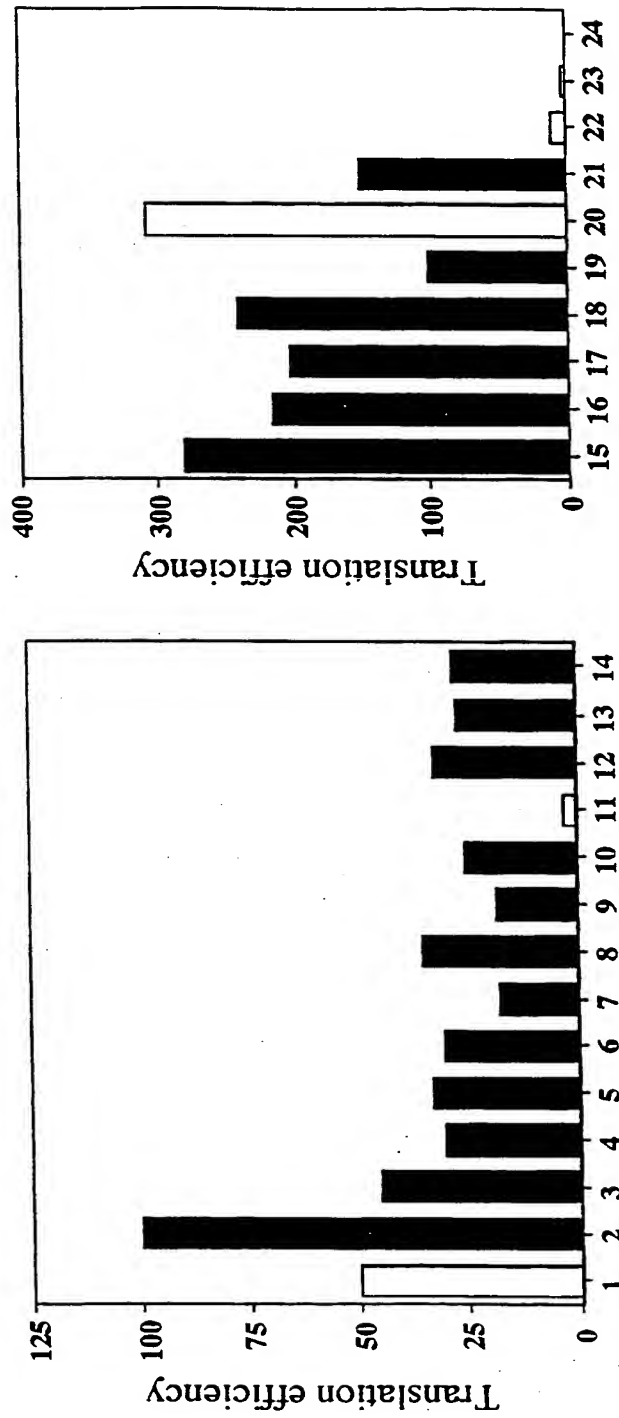
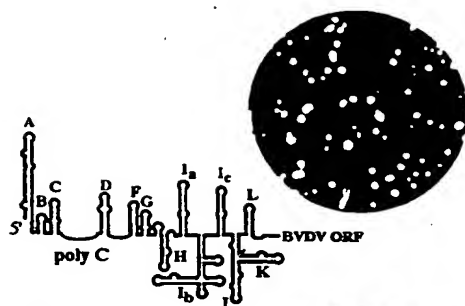
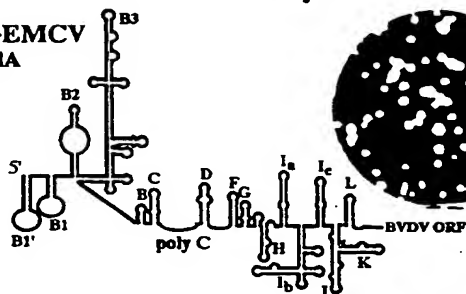
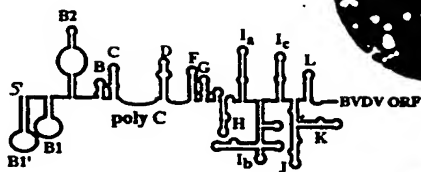
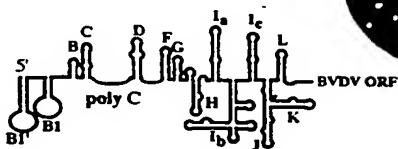


FIGURE 3



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5'EMCV

BVDV+EMCV  
delABVDV+EMCV  
delB3ABVDV+EMCV  
delB2B3A

Translation efficiency (% of BVDV)	Infectivity (PFU/ $\mu$ g of RNA)	Virus titer (PFU/ml)		Pseudorevertants
		24h	48h	
307	$2 \times 10^3$	$5 \times 10^2$	$3 \times 10^6$ (72h)	Yes
153	$3 \times 10^6$	$6 \times 10^6$	$6 \times 10^7$	Yes
280	$2 \times 10^6$	$5 \times 10^5$	$7 \times 10^7$	Yes
216	$4 \times 10^6$	$6 \times 10^5$	$5 \times 10^7$	Yes

FIGURE 4A

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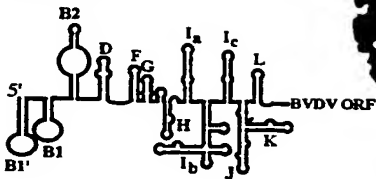
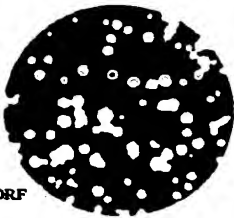
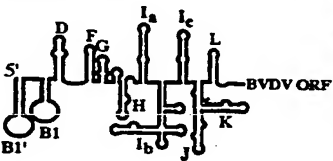
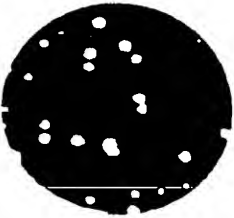
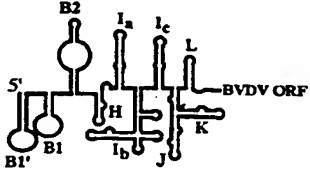
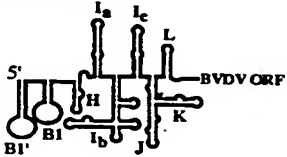
	Translation efficiency (% of BVDV)	Infectivity (PFU/ $\mu$ g of RNA)	Virus titer (PFU/ml)		Pseudorevertants
			24h	48h	
<b>BVDV+EMCV</b> <b>delB3ABC</b>  	202	$3 \times 10^6$	$8 \times 10^6$	$6 \times 10^7$	Yes
<b>BVDV+EMCV</b> <b>delB2B3ABC</b>  	240	$1 \times 10^6$	$1 \times 10^6$	$7 \times 10^7$	Yes
<b>BVDV+EMCV</b> <b>delB3A-H</b> 	12	0	<50	<50	No
<b>BVDV+EMCV</b> <b>delB2B3A-H</b> 	3	0	<50	<50	No

FIGURE 4B

5'HCV

5'EMCV

BVDV+HCV  
delB1B2B3

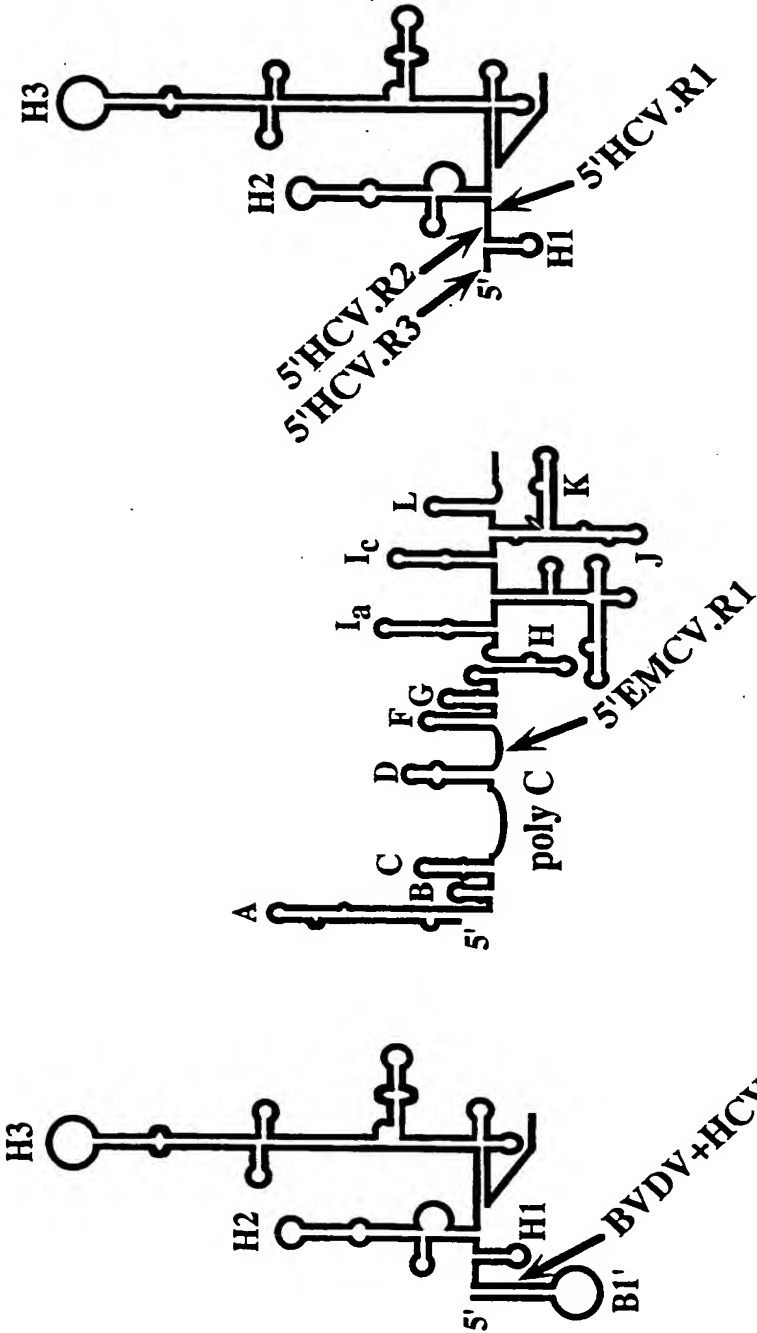


FIGURE 5A

B

BVDV+HCVdelB1B2B3

B1' < > H1 >  
BVDV+HCVd 1B1B2B3 guuacgagaauuagaaaaggcacucguauacguaCAUGGCACGUgccagccccucgugaggggc  
BVDV+HCVd 1B1B2B3.R1 guuacguaCAUGGCACGUgccagccccucgugaggggc

5'HCV

< H1 > < H2  
gccagccccucgugaggggcgacacuccaccaugaaucaucuccccucgugaggaac  
(G/A)UAUCAGAAUGCGAAUGCUGAacacuccaccaugaaucaucuccccucgugaggaac  
(G/A)UAUUGCAGUUUgccagccccucgugaggggcgacacuccaccaugaaucaucuccccucgugaggaac

5'EMCV

1 guugaaagccgggguggg..... 201 guuugucuauauguuuuuuuccaccuuuug  
5'EMCV Guuuguuuuuuuccaccuuuug  
5'EMCV.R1

FIGURE 5B

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A

5'HCV	gccagccccugauggggagacacuccaccaugaaucaucuccccugugaggaacu
5'HCV.R1orig	<u>GU</u> aaucacuccccugugaggaacu
5'HCV.R1cons	<u>GU</u> AUaaucacuccccugugaggaacu
5'HCV.R2orig	<u>GU</u> AUCAGAGUGCGAAUGCUGAACacuccaccaccaugaaucaucuccccugugaggaacu
5'HCV.R2cons	<u>GU</u> AVacacuccaccaugaaucaucuccccugugaggaacu
5'HCV.R3orig	<u>GU</u> AUUGCAGUUUgccagccccugauggggagacacuccaccaccaugaaucaucuccccugugaggaacu
5'HCV.R3cons	<u>GU</u> AVgccagccccugauggggagacacuccaccaugaaucaucuccccugugaggaacu

FIGURE 6A

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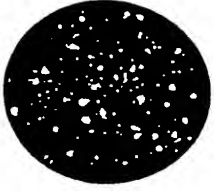
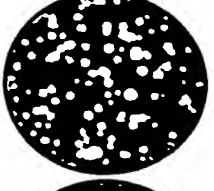
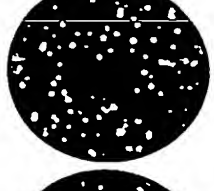
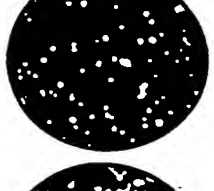


		Translation efficiency (% of BVDV)	Infectivity (PFU/ $\mu$ g of RNA)	Virus titer (PFU/ml)	
				24h	48h
<b>BVDV</b>		100	$4 \times 10^6$	$7 \times 10^7$	$1 \times 10^8$
<b>5'HCV.R1orig</b> (5' -GUA A)		45	$4 \times 10^5$	$2 \times 10^3$	$2 \times 10^5$
<b>5'HCV.R1cons</b> (5' -GUAU)		29	$3 \times 10^6$	$4 \times 10^7$	$5 \times 10^7$
<b>5'HCV.R2orig</b> (5' -GUAUCAGAAGUGCGAAUGCUGA)		17	$2 \times 10^6$	$7 \times 10^6$	$5 \times 10^7$
<b>5'HCV.R2cons</b> (5' -GUAU)		35	$3 \times 10^6$	$2 \times 10^7$	$4 \times 10^7$
<b>5'HCV.R3orig</b> (5' -GUAUUGCAGUUU)		33	$3 \times 10^6$	$4 \times 10^7$	$5 \times 10^7$
<b>5'HCV.R3cons</b> (5' -GUAU)		30	$3 \times 10^6$	$1 \times 10^7$	$6 \times 10^7$

FIGURE 6B

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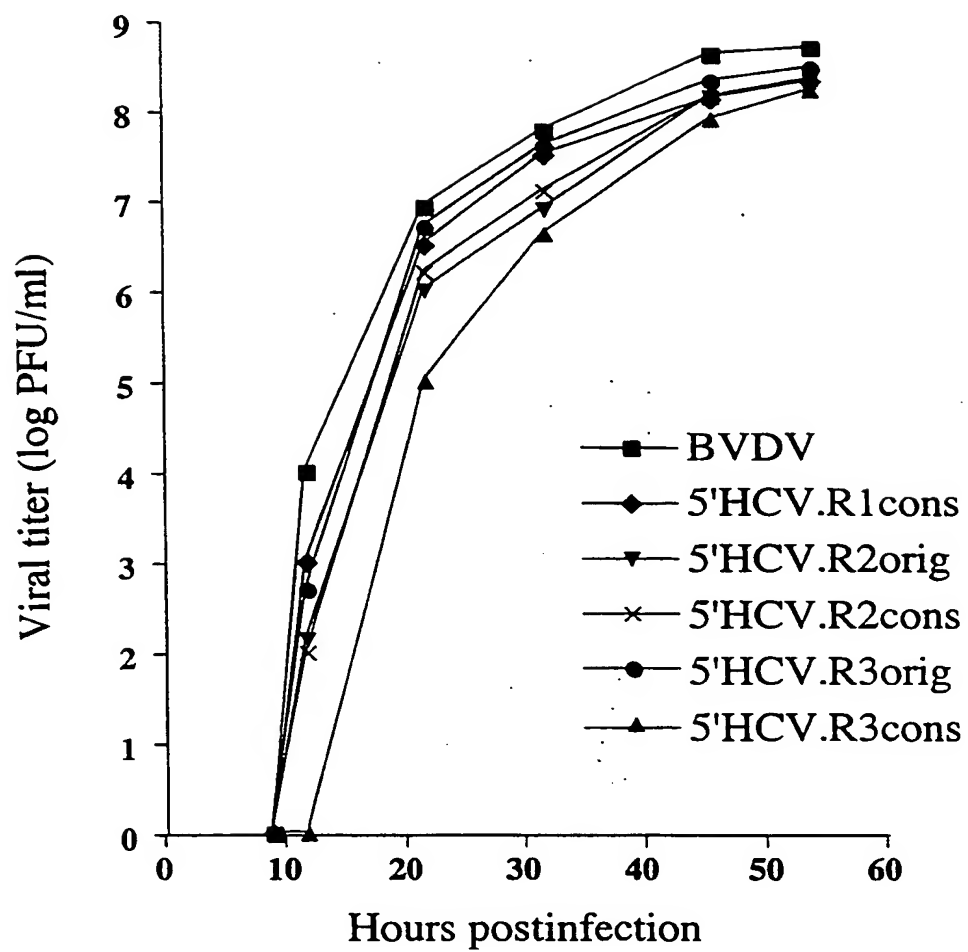


FIGURE 7

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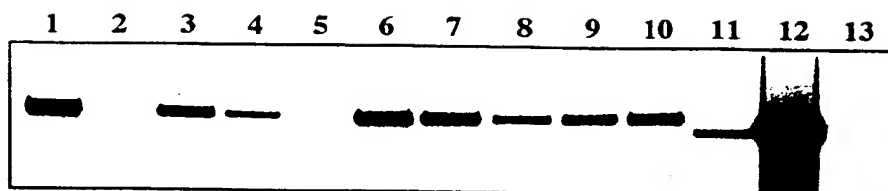
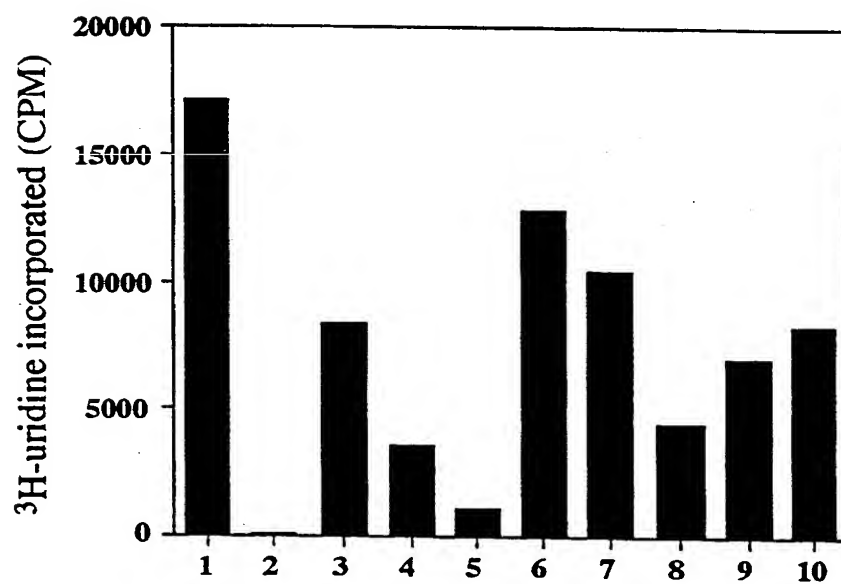
**A****B**

FIGURE 8



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## pACNR/BVD NADL-Xba\* -&gt; Graphic Map

DNA sequence 15065 bp gtatacgagaat ... cgactcactata circular

pACNR/BVD NADL-Xba = HaeII and XhoI digest of pACNR/BVD NADL ligated to  
 HaeII and XhoI digest of pACNR1180/DraIII-/BVD5'  
 8/27 corrected nt 12136 G to C to give HpaI site.

Co

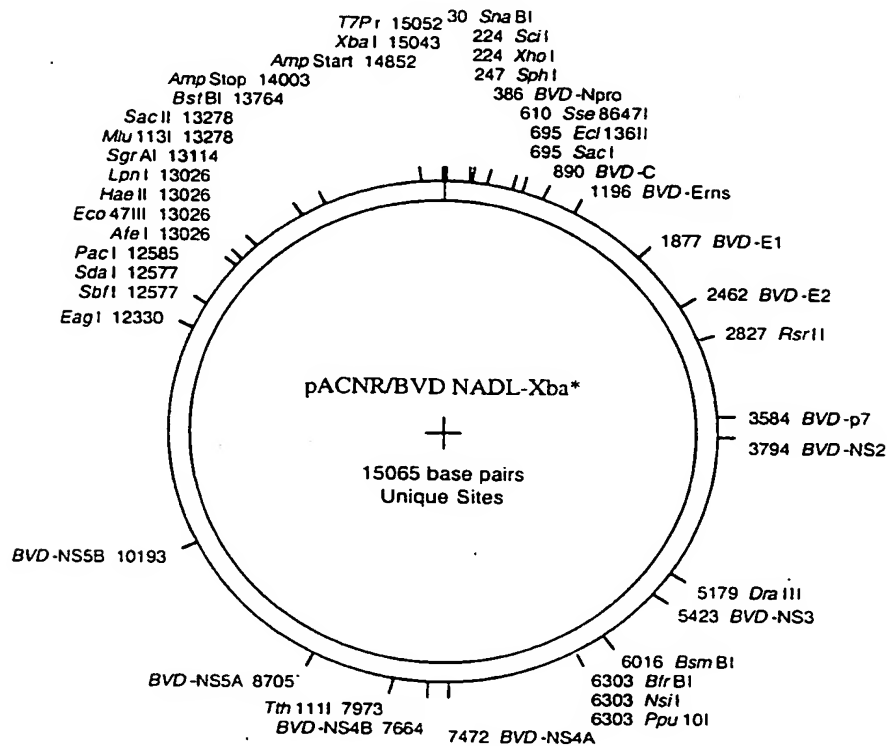


FIGURE 9

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## pACNR/BVD NADL-Xba\* -&gt; Genes

DNA sequence 15065 b.p. gtatacagagaat ... cgactcactata circular

pACNR/BVD NADL-Xba = HaeII and XhoI digest of pACNR/BVD NADL ligated to  
HaeII and XhoI digest of pACNR1180/DraIII-/BVD5'  
8/27 corrected nt 12136 G to C to give HpaI site.

Co

```

1 gtatacagagaattagaaaaggcactcgtatcgtattgggcaattaaaaataataattaggcctagggaacaaatccctc 80
81 tcagcgaaggccgaaaagaggcttagccatgcccttagtaggactagcataatgagggggtagcaacagtggtagttcg 160
161 ttggatggccttaagccctgagtacagggtagtcgtcagtggttcgacgccttggaataaaggctcgcagatgccacgtg 240
241 acgagggcacatgcccacacatcttaacctgagcgggggtcgcccagggtaaaagcagttttaaccgactgttacgaata 320
321 cagcctgataggggtgctgcagaggccactgtattgctactaaaaatctctgctgtacatggcac ATG GAG TTG 394
1 M E L 3
395 ATC ACA AAT GAA CTT TTA TAC AAA ACA TAC AAA CAA AAA CCC GTC GGG GTG GAG GAA CCT 454
4 I T N E L L Y K T Y K Q K P V G V E E P 23
455 GTT TAT GAT CAG GCA GGT GAT CCC TTA TTT GGT GAA AGG GGA GCA GTC CAC CCT CAA TCG 514
24 V Y D Q A G D P L F G E R G A V H P Q S 43
515 ACG CTA AAG CTC CCA CAC AAG AGA GGG GAA CGC GAT GTT CCA ACC AAC TTG GCA TCC TTA 574
44 T L K L P H K R G E R D V P T N L A S L 63
575 CCA AAA AGA GGT GAC TGC AGG TCG GGT AAT AGC AGA GGA CCT GTG AGC GGG ATC TAC CTG 634
64 P K R G D C R S G N S R G P V S G I Y L 83
635 AAG CCA GGG CCA CTA TTT TAC CAG GAC TAT AAA GGT CCC GTC TAT CAC AGG GCC CCG CTG 694
84 K P G P L F Y Q D Y K G P V Y H R A P L 103
695 GAG CTC TTT GAG GAG GGA TCC ATG TGT GAA ACG ACT AAA CGG ATA GGG AGA GTA ACT GGA 754
104 E L F E E G S M C E T T K R I G R V T G 123
755 AGT GAC GGA AAG CTG TAC CAC ATT TAT GTG TGT ATA GAT GGA TGT ATA ATA AAA AGT 814
124 S D G K L Y H I Y V C I D G C I I I K S 143
815 GCC ACG AGA AGT TAC CAA AGG GTG TTC AGG TGG GTC CAT AAT AGG CTT GAC TGC CCT CTA 874
144 A T R S Y Q R V F R W V H N R L D C P L 163
875 TGG GTC ACA ACT TGC TCA GAC ACG AAA GAA GAG GGA GCA ACA AAA AAG AAA ACA CAG AAA 934
164 W V T T C S D T K E E G A T K K K T Q K 183
935 CCC GAC AGA CTA GAA AGG GGG AAA ATG AAA ATA GTG CCC AAA GAA TCT GAA AAA GAC AGC 994
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204 K T K P P D A T I V V E G V K Y Q V R K 223
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244 Q E S R K K L E K A L L A W A I I A I V 263
1175 TTG TTT CAA GTT ACA ATG GGA GAA AAC ATA ACA CAG TGG AAC CTA CAA GAT AAT GGG ACG 1234
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1235 GAA GGG ATA CAA CGG GCA ATG TTC CAA AGG GGT GTG AAT AGA AGT TTA CAT GGA ATC TGG 1294
284 E G I Q R A M F Q R G V N R S L H G I W 303
1295 CCA GAG AAA ATC TGT ACT GGT GTC CCT TCC CAT CTA GCC ACC GAT ATA GAA CTA AAA ACA 1354
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1415 CAT GAG TGG AAC AAG CAT GGT TGG TGC AAC TGG TAC AAT ATT GAA CCC TGG ATT CTA GTC 1474
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1475 ATG AAT AGA ACC CAA GCC AAT CTC ACT GAG GGA CAA CCA CCA AGG GAG TGC GCA GTC ACT 1534
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384 C R Y D R A S D L N V V T Q A R D S P T 403
1595 CCC TTA ACA GGT TGC AAG AAA GGA AAG AAC TTC TCC TTT GCA GGC ATA TTG ATG CGG GGC 1654
404 P L T G C K K G K N F S F A G I L M R G 423

```

FIGURE 10-1

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1655 CCC TGC AAC TTT GAA ATA GCT GCA AGT GAT GTA TTA TTC AAA GAA CAT GAA CGC ATT AGT 1714  
 424 P C N F E I A A S D V L F K E H E R I S 443  
 1715 ATG TTC CAG GAT ACT ACT CTT TAC CTT GTT GAC GGG TTG ACC AAC TCC TTA GAA GGT GCC 1774  
 444 M F Q D T T L Y L V D G L T N S L E G A 463  
 1775 AGA CAA GGA ACC GCT AAA CTG ACA ACC TGG TTA GGC AAG CAG CTC GGG ATA CTA GGA AAA 1834  
 464 R Q G T A K L T T W L G K Q L G I L G K 483  
 1835 AAG TTG GAA AAC AAG AGT AAG ACG TGG TTT GGA GCA TAC GCT GCT TCC CCT TAC TGT GAT 1894  
 484 K L E N K S K T W F G A Y A A S P Y C D 503  
 1895 GTC GAT CGC AAA ATT GGC TAC ATA TGG TAT ACA AAA AAT TGC ACC CCT GCC TGC TTA CCC 1954  
 504 V D R K I G Y I W Y T K N C T P A C L P 523  
 1955 AAG AAC ACA AAA ATT GTC GGC CCT GGG AAA TTT GAC ACC AAT GCA GAG GAC GGC AAG ATA 2014  
 524 K N T K I V G P K F D T N A E D G 543  
 2015 TTA CAT GAG ATG GGG GGT CAC TTG TCG GAG GTA CTA CTA CTT TCT TTA GTG GTG CTG TCC 2074  
 544 L H E M G G H L S E V L L L S L V V L S 563  
 2075 GAC TTC GCA CCG GAA ACA GCT AGT GTA ATG TAC CTA ATC CTA CAT TTT TCC ATC CCA CAA 2134  
 564 D F A P E T A S V M Y L I L H F S I P Q 583  
 2135 AGT CAC GTT GAT GTA ATG GAT TGT GAT AAG ACC CAG TTG AAC CTC ACA GTG GAG CTG ACA 2194  
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 2195 ACA GCT GAA GTA ATA CCA GGG TCG GTC TGG AAT CTA GGC AAA TAT GTA TGT ATA AGA CCA 2254  
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 624 N W W P Y E T T V V L A F E E V S Q V V 643  
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 644 K L V L R A L R D L T R I W N A A T T T 663  
 2375 GCT TTT TTA GTA TGC CTT GTT AAG ATA GTC AGG GGC CAG ATG GTA CAG GGC ATT CTG TGG 2434  
 664 A F L V C L V K I V R G Q M V Q G I L W 683  
 2435 CTA CTA TTG ATA ACA GGG GTA CAA GGG CAC TTG GAT TGC AAA CCT GAA TTC TCG TAT GCC 2494  
 684 L L L I T G V Q G H L D C K P E F S Y A 703  
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 704 I A K D E R I G Q L G A E G L T T T W K 723  
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 804 V R G K F N T T L L N G P A F Q M V C P 823  
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 3035 GGA GAC CAA CTA CTA TAC AAA GGG GGC GCT ATT GAA TCT TGC AAG TGG TGT GGC TAT CAA 3094  
 884 G D Q L L Y K G S I E S C K W C G Y Q 903  
 3095 TTT AAA GAG AGT GAG GGA CTA CCA CAC TAC CCC ATT GGC AAG TGT AAA TTG GAG AAC GAG 3154  
 904 F K E S E G L P H Y P I G K C K L E N E 923  
 3155 ACT GGT TAC AGG CTA GTA GAC AGT ACC TCT TGC AAT AGA GAA GGT GTG GCC ATA GTA CCA 3214  
 924 T G Y R L V D S T S C N R E G V A I V P 943  
 3215 CAA GGG ACA TTA AAG TGC AAG ATA GGA AAA ACA ACT GTA CAG GTC ATA GCT ATG GAT ACC 3274  
 944 Q G T L K C I G K T V Q V I A M D T 963  
 3275 AAA CTC GGA CCT ATG CCT TGC AGA CCA TAT GAA ATC ATA TCA AGT GAG GGC CCT GTA GAA 3334  
 964 K L G P M P C R P Y E I I S S E G P V E 983  
 3335 AAG ACA GCG TGT ACT TTC AAC TAC ACT AAG ACA TTA AAA AAT AAG TAT TTT GAG CCC AGA 3394  
 984 K T A C T F N Y T K T L K N K Y F E P R 1003

FIGURE 10-2

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3395 GAC AGC TAC TTT CAG CAA TAC ATG CTA AAA GGA GAG TAT CAA TAC TGG TTT GAC CTG GAG 3454  
1004 D S Y F Q Q Y M L K G E Y Q Y W F D L E 1023

3455 GTG ACT GAC CAT CAC CGG GAT TAC TTC GCT GAG TCC ATA TTA GTG GTG GTA GTA GCC CTC 3514  
1024 V T D H H R D Y F A E S I L V V V V A L 1043

3515 TTG GGT GGC AGA TAT GTA CTT TGG TTA CTG GTT ACA TAC ATG GTC TTA TCA GAA CAG AAG 3574  
1044 L G G R Y V L W L L V T Y M V L S E Q K 1063

3575 GCC TTA GGG ATT CAG TAT GGA TCA GGG GAA GTG GTG ATG ATG GGC AAC TTG CTA ACC CAT 3634  
1064 A L G I Q Y G S G E V V M M G N L L T H 1083

3635 AAC AAT ATT GAA GTG GTG ACA TAC TTC TTG CTG CTG TAC CTA CTG CTG AGG GAG GAG AGC 3694  
1084 N N I E V V T Y F L L L Y L L L R E E S 1103

3695 GTA AAG AAG TGG GTC TTA CTC TTA TAC CAC ATC TTA GTG GTA CAC CCA ATC AAA TCT GTA 3754  
1104 V K K I W V L L L Y H P I K S V 1123

3755 ATT GTG ATC CTA CTG ATG ATT GGG GAT GTG GTA AAG GCC GAT TCA GGG GGC CAA GAG TAC 3814  
1124 I V I L L M I G D V V K A D S G G Q E Y 1143

3815 TTG GGG AAA ATA GAC CTC TGT TTT ACA ACA GTA GTA CTA ATC GTC ATA GGT TTA ATC ATA 3874  
1144 L G K I D L C F T T V V L I V I G L I I 1163

3875 GCC AGG CGT GAC CCA ACT ATA GTG CCA CTG GTA ACA ATA ATG GCA GCA CTG AGG GTC ACT 3934  
1164 A R R D P T I V L V T I M A A L R V T 1183

3935 GAA CTG ACC CAC CAG CCT GGA GTT GAC ATC GCT GTG GCG GTC ATG ACT ATA ACC CTA CTG 3994  
1184 E L T H Q P G V D I A V A V M T I T L L 1203

3995 ATG GTT AGC TAT GTG ACA GAT TAT TTT AGA TAT AAA AAA TGG TTA CAG TGC ATT CTC AGC 4054  
1204 M V S Y V T D Y F R Y K K W L Q C I L S 1223

4055 CTG GTA TCT GCG GTG TTC TTG ATA AGA AGC CTA ATA TAC CTA GGT AGA ATC GAG ATG CCA 4114  
1224 L V S A V F L I R S L I Y L G R I E M P 1243

4115 GAG GTA ACT ATC CCA AAC TGG AGA CCA CTA ACT TTA ATA CTA TTA TAT TTG ATC TCA ACA 4174  
1244 E V T I P N W R P L T L I L L Y L I S T 1263

4175 ACA ATT GTA ACG AGG TGG AAG GTT GAC GTG GCT GGC CTA TTG TTG CAA TGT GTG CCT ATC 4234  
1264 T I V T R W K V D V A G L L L Q C V P I 1283

4235 TTA TTG CTG GTC ACA ACC TTG TGG GCC GAC TTC TTA ACC CTA ATA CTG ATC CTG CCT ACC 4294  
1284 L L L V T T L W A D F L T L I L I L P T 1303

4295 TAT GAA TTG GTT AAA TTA TAC TAT CTG AAA ACT GTT AGG ACT GAT ATA GAA AGA AGT TGG 4354  
1304 Y E L V K L Y Y L K T V R T D I E R S W 1323

4355 CTA GGG GGG ATA GAC TAT ACA AGA GTT GAC TCC ATC TAC GAC GTT GAT GAG AGT GGA GAG 4414  
1324 L G G I D Y T R V D S I Y D V D E S G E 1343

4415 GGC GTA TAT CTT TTT CCA TCA AGG CAG AAA GCA CAG GGG AAT TTT TCT ATA CTC TTG CCC 4474  
1344 G V Y L F P S R Q K A Q G N F S I L L P 1363

4475 CTT ATC AAA GCA ACA CTG ATA AGT TGC GTC AGC AGT AAA TGG CAG CTA ATA TAC ATG AGT 4534  
1364 L I K A T L I S C V S S K W Q L I Y M S 1383

4535 TAC TTA ACT TTG GAC TTT ATG TAC TAC ATG CAC AGG AAA GTT ATA GAA GAG ATC TCA GGA 4594  
1384 Y L T L G F M Y Y M H R K V I E E I S G 1403

4595 GGT ACC AAC ATA ATA TCC AGG TTA GTG GCA GCA CTC ATA GAG CTG AAC TGG TCC ATG GAA 4654  
1404 G T N I I S R L V A A L I E L N W S M E 1423

4655 GAA GAG GAG AGC AAA GGC TTA AAG AAG TTT TAT CTA TTG TCT GGA AGG TTG AGA AAC CTA 4714  
1424 E E E S K G L K K F Y L L S G R L R N L 1443

4715 ATA ATA AAA CAT AAG GTA AGG AAT GAG ACC GTG GCT TCT TGG TAC GGG GAG GAG GAA GTC 4774  
1444 I I K H K V R N E T V A S W Y G E E E V 1463

4775 TAC GGT ATG CCA AAG ATC ATG ACT ATA ATC AAG GCC AGT ACA CTG AGT AAG AGC AGG CAC 4834  
1464 Y G M P K I M T I I K A S T L S K S R H 1483

4835 TGC ATA ATA TGC ACT GTA TGT GAG GGC CGA GAG TGG AAA GGT GGC ACC TGC CCA AAA TGT 4894  
1484 C I I C T V C E G R E W K G G T C P K C 1503

4895 GGA CGC CAT GGG AAG CCG ATA ACG TGT GGG ATG TCG CTA GCA GAT TTT GAA GAA AGA CAC 4954  
1504 G R H G K P I T C G M S L A D F E E R H 1523

4955 TAT AAA AGA ATC TTT ATA AGG GAA GGC AAC TTT GAG GGT ATG TGC AGC CGA TGC CAG GGA 5014  
1524 Y K R I F I R E G N F E G M C S R C Q G 1543

5015 AAG CAT AGG AGG TTT GAA ATG GAC CGG GAA CCT AAG AGT GCC AGA TAC TGT GCT GAG TGT 5074  
1544 K H R R F E M D R E P K S A R Y C A E C 1563

5075 AAT AGG CTG CAT CCT GCT GAG GAA GGT GAC TTT TGG GCA GAG TCG AGC ATG TTG GGC CTC 5134  
1564 N R L H P A E E G D F W A E S S M L G L 1583

FIGURE 10-3

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5135 AAA ATC ACC TAC TTT GCG CTG ATG GAT GGA AAG GTG TAT GAT ATC ACA GAG TGG GCT GGA 5194  
1584 K I T Y F A L M D G K V Y D I T E W A G 1603

5195 TGC CAG CGT GTG GGA ATC TCC CCA GAT ACC CAC AGA GTC CCT TGT CAC ATC TCA TTT GGT 5254  
1604 C Q R V G I S P D T H R V P C H I S F G 1623

5255 TCA CGG ATG CCT TTC AGG CAG GAA TAC AAT GGC TTT GTA CAA TAT ACC GCT AGG GGG CAA 5314  
1624 S R M P F R Q E Y N G F V Q Y T A R G G O 1643

5315 CTA TTT CTG AGA AAC TTG CCC GTA CTG GCA ACT AAA GTA AAA ATG CTC ATG GTA GGC AAC 5374  
1644 L F L R N L P V L A T K V K M L M V G N 1663

5375 CTT GGA GAA GAA ATT GGT AAT CTG GAA CAT CTT GGG TGG ATC CTA AGG GGG CCT GCC GTG 5434  
1664 L G E E I G N L E H L G W I L R G P A V 1683

5435 TGT AAG AAG ATC ACA GAG CAC GAA AAA TGC CAC ATT AAT ATA CTG GAT AAA CTA ACC GCA 5494  
1684 C K K I T E H E K C H I N I L D K L T A 1703

5495 TTT TTC GGG ATC ATG CCA AGG GGG ACT ACA CCC AGA GCC CCG GTG AGG TTC CCT ACG AGC 5554  
1704 F F G I M P R G T T P R A P V R F P T S 1723

5555 TTA CTA AAA GTG AGG AGG GGT CTG GAG ACT GCC TGG GCT TAC ACA CAC CAA GGC GGG ATA 5614  
1724 L L K V R R G L E T A W A Y T H Q G G I 1743

5615 AGT TCA GTC GAC CAT GTA ACC GCC GGA AAA GAT CTA CTG GTC TGT GAC AGC ATG GGA CGA 5674  
1744 S S V D H V T A G K D L L V C D S M G R 1763

5675 ACT AGA GTG GTT TGC CAA AGC AAC AAC AGG TTG ACC GAT GAG ACA GAG TAT GGC GTC AAG 5734  
1764 T R V V C Q S N N R L T D E T E Y G V K 1783

5735 ACT GAC TCA GGG TGC CCA GAC GGT GCC AGA TGT TAT GTG TTA AAT CCA GAG GCC GTT AAC 5794  
1784 T D S G C P D G A R C Y V L N P E A V N 1803

5795 ATA TCA GGA TCC AAA GGG GCA GTC GTT CAC CTC CAA AAG ACA GGT GGA GAA TTC ACG TGT 5854  
1804 I S G S K G A V V H L Q K T G G E F T C 1823

5855 GTC ACC GCA TCA GGC ACA CCG GCT TTC TTC GAC CTA AAA AAC TTG AAA GGA TGG TCA GGC 5914  
1824 V T A S G T P A F F D L K N L K G W S G 1843

5915 TTG CCT ATA TTT GAA GCC TCC AGC GGG AGG GTT GGC AGA GTC AAA GTA GGG AAG AAT 5974  
1844 L P I F E A S S G R V V G R V K V G K N 1863

5975 GAA GAG TCT AAA CCT ACA AAA ATA ATG AGT GGA ATC CAG ACC GTC TCA AAA AAC AGA GCA 6034  
1864 E E S K P T K I M S G I Q T V S K N R A 1883

6035 GAC CTG ACC GAG ATG GTC AAG AAG ATA ACC AGC ATG AAC AGG GGA GAC TTC AAG CAG ATT 6094  
1884 D L T E M V K K I T S M N R G D F K Q I 1903

6095 ACT TTG GCA ACA GGG GCA GGC AAA ACC ACA GAA CTC CCA AAA GCA GTT ATA GAG GAG ATA 6154  
1904 T L A T G A G K T T E L P K A V I E I 1923

6155 GGA AGA CAC AGA AGA GTA TTA GTT CTT ATA CCA TTA AGG GCA GCG GCA GAG TCA GTC TAC 6214  
1924 G R H K R V L V L I P L R A A A E S V Y 1943

6215 CAG TAT ATG AGA TTG AAA CAC CCA AGC ATC TCT TTT AAC CTA AGG ATA GGG GAC ATG AAA 6274  
1944 Q Y M R L K H P S I S F N L R I G D M K 1963

6275 GAG GGG GAC ATG GCA ACC GGG ATA ACC TAT GCA TCA TAC GGG TAC TTC TGC CAA ATG CCT 6334  
1964 E G D M A T G I T Y A S Y G Y F C Q M P 1983

6335 CAA CCA AAG CTC AGA GCT GCT ATG GTA GAA TAC TCA TAC ATA TTC TTA GAT GAA TAC CAT 6394  
1984 Q P K L R A A M V E Y S Y I F L D E Y H 2003

6395 TGT GCC ACT CCT GAA CAA CTG GCA ATT ATC GGG AAG ATC CAC AGA TTT TCA GAG AGT ATA 6454  
2004 C A T P E Q L A I I G K I H R F S E S I 2023

6455 AGG GTT GTC GCC ATG ACT GCC ACG CCA GCA GGG TCG GTG ACC ACA ACA GGT CAA AAG CAC 6514  
2024 R V V A M T A T P A G S V T T T G Q K H 2043

6515 CCA ATA GAG GAA TTC ATA GCC CCC GAG GTA ATG AAA GGG GAG GAT CTT GGT AGT CAG TTC 6574  
2044 P I E E F I A P E V M K G E D L G S Q F 2063

6575 CTT GAT ATA GCA GGG TTA AAA ATA CCA GTG GAT GAG ATG AAA GGC AAT ATG TTG GTT TTT 6634  
2064 L D I A G L K I P V D E M K G N M L V F 2083

6635 GTA CCA ACG AGA AAC ATG GCA GTA GAG GTA GCA AAG AAG CTA AAA GCT AAG GGC TAT AAC 6694  
2084 V P T R N M A V E V A K K L K A K G Y N 2103

6695 TCT GGA TAC TAT TAC AGT GGA GAG GAT CCA GCC AAT CTG AGA GTT GTG ACA TCA CAA TCC 6754  
2104 S G Y Y Y S G E D P A N L R V V T S Q S 2123

6755 CCC TAT GTA ATC GTG GCT ACA AAT GCT ATT GAA TCA GGA GTG ACA CTA CCA GAT TTG GAC 6814  
2124 P Y V I V A T N A I E S G V T L P D L D 2143

6815 ACG GTT ATA GAC ACG GGG TTG AAA TGT GAA AAG AGG GTG AGG GTA TCA TCA AAG ATA CCC 6874  
2144 T V I D T G L K C E K R V R V S S K I P 2163

FIGURE 1024

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6875	TTC	ATC	GTA	ACA	GGC	CTT	AAG	AGG	ATG	GCC	GTG	ACT	GTG	GGT	GAG	CAG	GCG	CAG	CGT	AGG	6934
2164	F	I	V	T	G	L	K	R	M	A	V	T	V	G	E	Q	A	Q	R	R	2183
6935	GGC	AGA	GTA	GGT	AGA	GTG	AAA	CCC	GGG	AGG	TAT	TAT	AGG	AGC	CAG	GAA	ACA	GCA	ACA	GGG	6994
2184	G	R	V	G	R	V	K	P	G	R	Y	Y	R	S	Q	E	T	A	T	G	2203
6995	TCA	AAG	GAC	TAC	CAC	TAT	GAC	CTC	TTG	CAG	GCA	CAA	AGA	TAC	GGG	ATT	GAG	GAT	GGA	ATC	7054
2204	S	K	D	Y	H	Y	D	L	L	Q	A	Q	R	Y	G	I	E	D	G	I	2223
7055	AAC	GTG	ACG	AAA	TCC	TTT	AGG	GAG	ATG	AAT	TAC	GAT	TGG	AGC	CTA	TAC	GAG	GAG	GAC	AGC	7114
2224	N	V	T	K	S	F	R	E	M	N	Y	D	W	S	L	Y	E	E	D	S	2243
7115	CTA	CTA	ATA	ACC	CAG	CTG	GAA	ATA	CTA	AAT	AAT	CTA	CTC	ATC	TCA	GAA	GAC	TTG	CCA	GCC	7174
2244	L	L	I	T	Q	L	E	I	L	N	N	L	L	I	S	E	D	L	P	A	2263
7175	GCT	GTT	AAG	AAC	ATA	ATG	GCC	AGG	ACT	GAT	CAC	CCA	GAG	CCA	ATC	CAA	CTT	GCA	TAC	AAC	7234
2264	A	V	K	N	I	M	A	R	T	D	H	P	E	P	I	Q	L	A	Y	N	2283
7235	AGC	TAT	GAA	GTC	CAG	GTC	CCG	GTC	CTG	TTC	CCA	AAA	ATA	AGG	AAT	GGA	GAA	GTC	ACA	GAC	7294
2284	S	Y	E	V	Q	V	P	V	L	F	P	K	I	R	N	G	E	V	T	D	2303
7295	ACC	TAC	GAA	AAT	TAC	TCG	TTT	CTA	AAT	GCC	AGA	AAG	TTA	GGG	GAG	GAT	GTG	CCC	GTG	TAT	7354
2304	T	Y	E	N	Y	S	F	L	N	A	R	K	L	G	E	D	V	P	V	Y	2323
7355	ATC	TAC	GCT	ACT	GAA	GAT	GAG	GAT	CTG	GCA	GTT	GAC	CTC	TTA	GGG	CTA	GAC	TGG	CCT	GAT	7414
2324	I	Y	A	T	E	D	E	D	L	A	V	D	L	L	G	L	D	W	P	D	2343
7415	CCT	GGG	AAC	CAG	CAG	GTA	GTG	GAG	ACT	GGT	AAA	GCA	CTG	AAG	CAA	GTG	ACC	GGG	TTG	TCC	7474
2344	P	G	N	Q	Q	V	V	E	T	G	K	A	L	K	Q	V	T	G	L	S	2363
7475	TCG	GCT	GAA	AAT	GCC	CTA	CTA	GTG	GCT	TTA	TTT	GGG	TAT	GTG	GGT	TAC	CAG	GCT	CTC	TCA	7534
2364	S	A	E	N	A	L	L	V	A	L	F	G	Y	V	G	Y	Q	A	L	S	2383
7535	AAG	AGG	CAT	GTC	CCA	ATG	ATA	ACA	GAC	ATA	TAT	ACC	ATC	GAG	GAC	CAG	AGA	CTA	GAA	GAC	7594
2384	K	R	H	V	P	M	I	T	D	I	Y	T	I	E	D	Q	R	L	E	D	2403
7595	ACC	ACC	CAC	CTC	CAG	TAT	GCA	CCC	AAC	GCC	ATA	AAA	ACC	GAT	GGG	ACA	GAG	ACT	GAA	CTG	7654
2404	T	T	H	L	Q	Y	A	P	N	A	I	K	T	D	G	T	E	T	E	L	2423
7655	AAA	GAA	CTG	GCG	TCG	GGT	GAC	GTG	GAA	AAA	ATC	ATG	GGA	GCC	ATT	TCA	GAT	TAT	GCA	GCT	7714
2424	K	E	L	A	S	G	D	V	E	K	I	M	G	A	I	S	D	Y	A	A	2443
7715	GGG	GGA	CTG	GAG	TTT	GTT	AAA	TCC	CAA	GCA	GAA	AAG	ATA	AAA	ACA	GCT	CCT	TTG	TTT	AAA	7774
2444	G	G	L	E	F	V	K	S	Q	A	E	K	I	K	T	A	P	L	F	K	2463
7775	GAA	AAC	GCA	GAA	GCC	GCA	AAA	GGG	TAT	GTG	CAA	AAA	TTC	ATT	GAC	TCA	TTA	ATT	GAA	AAT	7834
2464	E	N	A	E	A	A	K	G	Y	V	Q	K	F	I	D	S	L	I	E	N	2483
7835	AAA	GAA	GAA	ATA	ATC	AGA	TAT	GGT	TTG	TGG	GGA	ACA	CAC	ACA	GCA	CTA	TAC	AAA	AGC	ATA	7894
2484	K	E	E	I	I	R	Y	G	L	W	G	T	H	T	A	L	Y	K	S	I	2503
7895	GCT	GCA	AGA	CTG	GGG	CAT	GAA	ACA	GCG	TTT	GCC	ACA	CTA	GTG	TTA	AAG	TGG	CTA	GCT	TTT	7954
2504	A	A	R	L	G	H	E	T	A	F	A	T	L	V	L	K	W	L	A	F	2523
7955	GGA	GGG	GAA	TCA	GTG	TCA	GAC	CAC	GTC	AAG	CAG	GCG	GCA	GTT	GAT	TTA	GTG	GTC	TAT	TAT	8014
2524	G	G	E	S	V	S	D	H	V	K	Q	A	A	V	D	L	V	V	Y	Y	2543
8015	GTG	ATG	AAT	AAG	CCT	TCC	TTC	CCA	GGT	GAC	TCC	GAG	ACA	CAG	CAA	GAA	GGG	AGG	CGA	TTC	8074
2544	V	M	N	K	P	S	F	P	G	D	S	E	T	Q	Q	E	G	R	R	F	2563
8075	GTC	GCA	AGC	CTG	TTC	ATC	TCC	GCA	CTG	GCA	ACC	TAC	ACA	TAC	AAA	ACT	TGG	AAT	TAC	CAC	8134
2564	V	A	S	L	F	I	S	A	L	A	T	Y	T	Y	K	T	W	N	Y	H	2583
8135	AAT	CTC	TCT	AAA	GTG	GTG	GAA	CCA	GCC	CTG	GCT	TAC	CTC	CCC	TAT	GCT	ACC	AGC	GCA	TTA	8194
2584	N	L	S	K	V	V	E	P	A	L	A	Y	L	P	Y	A	T	S	A	L	2603
8195	AAA	ATG	TTC	ACC	CCA	ACG	CGG	CTG	GAG	AGC	GTG	GTG	ATA	CTG	AGC	ACC	ACG	ATA	TAT	AAA	8254
2604	K	M	F	T	P	T	R	L	E	S	V	V	I	L	S	T	T	I	Y	K	2623
8255	ACA	TAC	CTC	TCT	ATA	AGG	AAG	GGG	AAG	AGT	GAT	GGA	TTG	CTG	GGT	ACG	GGG	ATA	AGT	GCA	8314
2624	T	Y	L	S	I	R	K	G	G	S	D	G	L	L	G	T	G	I	S	A	2643
8315	GCC	ATG	GAA	ATC	CTG	TCA	CAA	AAC	CCA	GTA	TCG	GTA	GGT	ATA	TCT	GTG	ATG	TTG	GGG	GTA	8374
2644	A	M	E	I	L	S	Q	N	P	V	S	V	G	I	S	V	M	L	G	V	2663
8375	GGG	GCA	ATC	GCT	GCG	CAC	AAC	GCT	ATT	GAG	TCC	AGT	GAA	CAG	AAA	AGG	ACC	CTA	CTT	ATG	8434
2664	G	A	I	A	A	H	N	A	I	E	S	S	E	Q	K	R	T	L	L	M	2683
8435	AAG	GTG	TTT	GTA	AAG	AAC	TTC	TTG	GAT	CAG	GCT	GCA	ACA	GAT	GAG	CTG	GTA	AAA	GAA	AAC	8494
2684	K	V	F	V	K	N	F	L	V	Q	A	A	T	D	E	L	V	K	E	N	2703
8495	CCA	GAA	AAA	ATT	ATA	ATG	GCC	TTA	TTT	GAA	GCA	GTC	CAG	ACA	ATT	GGT	AAC	CCC	CTG	AGA	8554
2704	P	E	K	I	I	M	A	L	F	E	A	V	Q	T	I	G	N	P	L	R	2723
8555	CTA	ATA	TAC	CAC	CTG	TAT	GGG	GTT	TAC	TAC	AAA	GGT	TGG	GAG	GCC	AAG	GAA	CTA	TCT	GAG	8614
2724	L	I	Y	H	L	Y	G	V	Y	Y	K	G	W	E	A	K	E	L	S	E	2743

FIGURE 10-5

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8615 AGG ACA GCA GGC AGA AAC TTA TTC ACA TTG ATA ATG TTT GAA GCC TTC GAG TTA TTA GGG 8674  
2744 R T A G R N L F T L I M F E A F E L L G 2763

8675 ATG GAC TCA CAA GGG AAA ATA AGG AAC CTG TCC GGA AAT TAC ATT TTG GAT TTG ATA TAC 8734  
2764 M D S Q G K I R N L S G N Y I L D L I Y 2783

8735 GGC CTA CAC AAG CAA ATC AAC AGA GGG CTG AAG AAA ATG GTA CTG GGG TGG GCC CCT GCA 8794  
2784 G L H K Q I N R G L K K M V L G W A P A 2803

8795 CCC TTT AGT TGT GAC TGG ACC CCT AGT GAC GAG AGG ATC AGA TTG CCA ACA GAC AAC TAT 8854  
2804 P F S C D W T P S D E R I R L P T D N Y 2823

8855 TTG AGG GTA GAA ACC AGG TGC CCA TGT GGC TAT GAG ATG AAA GCT TTC AAA AAT GTA GGT 8914  
2824 L R V E T R C P C G Y E M K A F K N V G 2843

8915 GGC AAA CTT ACC AAA GTG GAG GAG AGC GGG CCT TTC CTA TGT AGA AAC AGA CCT GGT AGG 8974  
2844 G K L T K V E E S G P F L C R N R G R 2863

8975 GGA CCA GTC AAC TAC AGA GTC ACC AAG TAT TAC GAT GAC AAC CTC AGA GAG ATA AAA CCA 9034  
2864 G P V N Y R V T K Y Y D D N L R E I K P 2883

9035 GTA GCA AAG TTG GAA GGA CAG GTA GAG CAC TAC TAC AAA GGG GTC ACA GCA AAA ATT GAC 9094  
2884 V A K L E G Q V E H Y Y K G V T A K I D 2903

9095 TAC AGT AAA GGA AAA ATG CTC TTG GCC ACT GAC AAG TGG GAG GTG GAA CAT GGT GTC ATA 9154  
2904 Y S K G K M L L A T D K W E V E H G V I 2923

9155 ACC AGG TTA GCT AAG AGA TAT ACT GGG GTC GGG TTC AAT GGT GCA TAC TTA GGT GAC GAG 9214  
2924 T R L A K R Y T G F N G A Y L G D E 2943

9215 CCC AAT CAC CGT GCT CTA GTG GAG AGG GAC TGT GCA ACT ATA ACC AAA AAC ACA GTA CAG 9274  
2944 P N H R A L V E R D C A T I T K N T V Q 2963

9275 TTT CTA AAA ATG AAG AAG GGG TGT GCG TTC ACC TAT GAC CTG ACC ATC TCC AAT CTG ACC 9334  
2964 F L K M K K G C A F T Y D L T I S N L T 2983

9335 AGG CTC ATC GAA CTA GTA CAC AGG AAC AAT CTT GAA GAG AAG GAA ATA CCC ACC GCT ACG 9394  
2984 R L I E L V H R N N L E E K E I P T A T 3003

9395 GTC ACC ACA TGG CTA GCT TAC ACC TTC GTG AAT GAA GAC GTA GGG ACT ATA AAA CCA GTA 9454  
3004 V T T W L A Y T F V N E D V G T I K P V 3023

9455 CTA GGA GAG AGA GTA ATC CCC GAC CCT GTA GTT GAT ATC AAT TTA CAA CCA GAG GTG CAA 9514  
3024 L G E R V I P D P V V D I N L Q P E V Q 3043

9515 GTG GAC ACG TCA GAG GTT GGG ATC ACA ATA ATT GGA AGG GAA ACC CTG ATG ACA ACG GGA 9574  
3044 V D T S E V G I T I I G R E T L M T T G 3063

9575 GTG ACA CCT GTC TTG GAA AAA GTA GAG CCT GAC GCC AGC GAC AAC CAA AAC TCG GTG AAG 9634  
3064 V T P L E K V E G P D A S D N Q N S V K 3083

9635 ATC GGG TTG GAT GAG GGT AAT TAC CCA GGG CCT GGA ATA CAG ACA CAT ACA CTA ACA GAA 9694  
3084 I G L D E G N Y P G P G I Q T H T L T E 3103

9695 GAA ATA CAC AAC AGG GAT GCG AGG CCC TTC ATC ATG ATC CTG GGC TCA AGG AAT TCC ATA 9754  
3104 E I H N R D A R P F I M I L G S R N S I 3123

9755 TCA AAT AGG GCA AAG ACT GCT AGA AAT ATA AAT CTG TAC ACA GGA AAT GAC CCC AGG GAA 9814  
3124 S N R A K T A R N I N L Y T G N D P R E 3143

9815 ATA CGA GAC TTG ATG GCT GCA GGG CGC ATG TTA GTA GTA GCA CTG AGG GAT GTC GAC CCT 9874  
3144 I R D L M A A G R M L V V A L R D V D P 3163

9875 GAG CTG TCT GAA ATG GTC GAT TTC AAG GGG ACT TTT TTA GAT AGG GAG GCC CTG GAG GCT 9934  
3164 E L S E M V D F K G T F L D R E A L E A 3183

9935 CTA AGT CTC GGG CAA CCT AAA CCG AAG CAG GTT ACC AAG GAA GCT GTT AGG AAT TTG ATA 9994  
3184 L S L G Q P K P K Q V T K E A V R N L I 3203

9995 GAA CAG AAA AAA GAT GTG GAG ATC CCT AAC TGG TTT GCA TCA GAT GAC CCA GTA TTT CTG 10054  
3204 E Q K K D V E I P N W F A S D D P V F L 3223

10055 GAA GTG GCC TTA AAA AAT GAT AAG TAC TAC TTA GTA GGA GAT GTT GGA GAG CTA AAA GAT 10114  
3224 E V A L K N D K Y Y L V G D V G E L K D 3243

10115 CAA GCT AAA GCA CTT GGG GCC ACG GAT CAG ACA AGA ATT ATA AAG GAG GTA GGC TCA AGG 10174  
3244 Q A K A L G A T D Q T R I I K E V G S R 3263

10175 ACG TAT GCC ATG AAG CTA TCT AGC TGG TTC CTC AAG GCA TCA AAC AAA CAG ATG AGT TTA 10234  
3264 T Y A M K L S S W F L K A S N K O M S L 3283

10235 ACT CCA CTG TTT GAG GAA TTG TTG CTA CGG TGC CCA CCT GCA ACT AAG AGC AAT AAG GGG 10294  
3284 T P L F E L L L R C P P A T K S N K G 3303

10295 CAC ATG GCA TCA GCT TAC CAA TTG GCA CAG GGT AAC TGG GAG CCC CTC GGT TGC GGG GTG 10354  
3304 H M A S A Y Q L A Q G N W E P L G C G V 3323

FIGURE 10-6

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10355 CAC CTA GGT ACA ATA CCA GCC AGA AGG GTG AAG ATA CAC CCA TAT GAA GCT TAC CTG AAG 10414  
3324 H L G T I P A R R V K I H P Y E A Y L K 3343

10415 TTG AAA GAT TTC ATA GAA GAA GAA GAG AAG AAA CCT AGG GTT AAG GAT ACA GTA ATA AGA 10474  
3344 L K D F I E E E E K K P R V K D T V I R 3363

10475 GAG CAC AAC AAA TGG ATA CTT AAA AAA ATA AGG TTT CAA GGA AAC CTC AAC ACC AAG AAA 10534  
3364 E H N K W I L K K I R F Q G N L N T K K 3383

10535 ATG CTC AAC CCG GGG AAA CTA TCT GAA CAG TTG GAC AGG GAG GGG CGC AAG AGG AAC ATC 10594  
3384 M L N P G K L S E Q L D R E G R K R N I 3403

10595 TAC AAC CAC CAG ATT GGT ACT ATA ATG TCA AGT GCA GGC ATA AGG CTG GAG AAA TTG CCA 10654  
3404 Y N H Q I G T I M S A G I R L E K L P 3423

10655 ATA GTG AGG GCC CAA ACC GAC ACC AAA ACC TTT CAT GAG GCA ATA AGA GAT AAG ATA GAC 10714  
3424 I V R A Q T D T K T F T H E A I R D K I D 3443

10715 AAG AGT GAA AAC CGG CAA AAT CCA GAA TTG CAC AAC AAA TTG TTG GAG ATT TTC CAC ACG 10774  
3444 K S E N R Q N P E L H N K L L E I F H T 3463

10775 ATA GCC CAA CCC ACC CTG AAA CAC ACC TAC GGT GAG GTG ACG TGG GAG CAA CTT GAG GCG 10834  
3464 I A Q P T L K H T Y G E V T W E Q L E A 3483

10835 GGG ATA AAT AGA AAG GGG GCA GCA GGC TTC CTG GAG AAG AAG AAC ATC GGA GAA GTA TTG 10894  
3484 G I N R K G G GCA GCA GGC TTC CTG GAG AAG AAG AAC ATC GGA GAA GTA TTG 3503

10895 GAT TCA GAA AAG CAC CTG GTA GAA CAA TTG GTC AGG GAT CTG AAG GCC GGG AGA AAG ATA 10954  
3504 D S E K H L V E Q L V R D L K A G R K I 3523

10955 AAA TAT TAT GAA ACT GCA ATA CCA AAA AAT GAG AAG AGA GAT GTC AGT GAT GAC TGG CAG 11014  
3524 K Y Y E T A I P K N E K R D V S D D W Q 3543

11015 GCA GGG GAC CTG GTG GTT GAG AAG AGG CCA AGA GTT ATC CAA TAC CCT GAA GCC AAG ACA 11074  
3544 A G D L V V E K R P R V I Q Y P E A K T 3563

11075 AGG CTA GCC ATC ACT AAG GTC ATG TAT AAC TGG GTG AAA CAG CAG CCC GTT GTG ATT CCA 11134  
3564 R L A I T K V M Y N W V K Q P V V I P 3583

11135 GGA TAT GAA GGA AAG ACC CCC TTG TTC AAC ATC TTT GAT AAA GTG AGA AAG GAA TGG GAC 11194  
3584 G Y E G K T P L F N I F D K V R K E W D 3603

11195 TCG TTC AAT GAG CCA GTG GCC GTA AGT TTT GAC ACC AAA GCC TGG GAC ACT CAA GTG ACT 11254  
3604 S F N E P V A V S F D T K A W D T Q V T 3623

11255 AGT AAG GAT CTG CAA CTT ATT GGA GAA ATC CAG AAA TAT TAC TAT AAG AAG GAG TGG CAC 11314  
3624 S K D L Q L I G E I Q K Y Y K K E W H 3643

11315 AAG TTC ATT GAC ACC ATC ACC GAC CAC ATG ACA GAA GTA CCA GTT ATA ACA GCA GAT GGT 11374  
3644 K F I D T I T D H M T E V P V I T A D G 3663

11375 GAA GTA TAT ATA AGA AAT GGG CAG AGA GGG AGC GGC CAG CCA GAC ACA AGT GCT GGC AAC 11434  
3664 E V Y I R N G Q R G S G Q P D T S A G N 3683

11435 AGC ATG TTA AAT GTC CTG ACA ATG ATG TAC GGC TTC TGC GAA AGC ACA GGG GTA CCG TAC 11494  
3684 S M L N V L T M M Y G F C E S T G V P Y 3703

11495 AAG AGT TTC AAC AGG GTG GCA AGG ATC CAC GTC TGT GGG GAT GAT GGC TTC TTA ATA ACT 11554  
3704 K S F N R V A R I H V C G D D G F L I T 3723

11555 GAA AAA GGG TTA GGG CTG AAA TTT GCT AAC AAA GGG ATG CAG ATT CTT CAT GAA GCA GGC 11614  
3724 E K G L G L K F A N K G M Q I L H E A G 3743

11615 AAA CCT CAG AAG ATA ACG GAA GGG GAA AAG ATG AAA GTT GCC TAT AGA TTT GAG GAT ATA 11674  
3744 K P Q K I T E G E K M K V A Y R F E D I 3763

11675 GAG TTC TGT TCT CAT ACC CCA GTC CCT GTT AGG TGG TCC GAC AAC ACC AGT AGT CAC ATG 11734  
3764 E F C S H T P V P V R W S D N T S H M 3783

11735 GCC GGG AGA GAC ACC GCT GTG ATA CTA TCA AAG ATG GCA ACA AGA TTG GAT TCA AGT GGA 11794  
3784 A G R D T A V I L S K M A T R L D S S G 3803

11795 GAG AGG GGT ACC ACA GCA TAT GAA AAA GCG GTA GCC TTC AGT TTC TTG CTG ATG TAT TCC 11854  
3804 E R G T T A Y E K A V A F S F L L M Y S 3823

11855 TGG AAC CCG CTT GTT AGG AGG ATT TGC CTG TTG GTC CTT TCG CAA CAG CCA GAG ACA GAC 11914  
3824 W N P L V R R I C L L V L S Q Q P E T D 3843

11915 CCA TCA AAA CAT GCC ACT TAT TAT TAC AAA GGT GAT CCA ATA GGG GCC TAT AAA GAT GTA 11974  
3844 P S K H A T Y Y Y K A G D P I G A Y K D V 3863

11975 ATA GGT CCG AAT CTA AGT GAA CTG AAG AGA ACA GGC TTT GAG AAA TTG GCA AAT CTA AAC 12034  
3864 I G R N L S E L K R T G F E K L A N L N 3883

12035 CTA AGC CTG TCC ACG TTG GGG ATC TGG ACT AAG CAC ACA AGC AAA AGA ATA ATT CAG GAC 12094  
3884 L S L S T L G I W T K H T S K R I I Q D 3903

FIGURE 10-7



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12095 TGT GTT GCC ATT GGG AAA GAA GAG GGC AAC TGG CTA GTT AAC GCC GAC AGG CTG ATA TCC 12154  
3904 C V A I G K E E G N W L V N A D R L I S 3923

12155 AGC AAA ACT GGC CAC TTA TAC ATA CCT GAT AAA GGC TTT ACA TTA CAA GGA AAG CAT TAT 12214  
3924 S K T G H L Y I P D K G F T L Q G K H Y 3943

12215 GAG CAA CTG CAG CTA AGA ACA GAG ACA AAC CCG GTC ATG GGG GTT GGG ACT GAG AGA TAC 12274  
3944 E Q L Q L R T E T N P V M G V G T E R Y 3963

12275 AAG TTA GGT CCC ATA GTC AAT CTG CTG CTG AGA AGG TTG AAA ATT CTG CTC ATG ACG GCC 12334  
3964 K L G P I V N L L L R R L K I L L M T A 3983

12335 GTC GGC GTC AGC AGC TGA gacaaaatgtatatattgttaaataaattaatccatgtacatagtgtatataaatat 12408  
3984 V G V S S . 3989

12409 agttgggaccgtccacctcaagaagacgacacgccccaacacgcacagctaacacagtagtcaagattatctacctcaagat 12488

12489 aacactacatttaatagcacacagcacttttagctgtatgaggatacgcgccgacgtctatagttggactaggggaagacctct 12568

12569 aacagcccccctgcagggttaattaaactagtgggaatacgcggggtatgccgcgttttagcatattgacgacccaattctca 12648

12649 tgtttgacagcttatcatcgtcgcagcaagacgtttcccggtgaatatgggtcataaacaccccttctgattactgtttatgt 12728

12729 aagcagacagttttattgttcatgatgatataatttttatcttgcgaatgtaacatcagagattttgagacacgtgggtt 12808

12809 tgttgaataaaatcgaacttttgcgtgagttgaaggatcagatcacgcacatcttcccgacaacgcagaccgttccgtggcaaa 12888

12889 gcaaaagtccaaaatcaccaactgggtccacctacaacaaagctctcatcaaccgtgggtccctcactttctggctggatg 12968

12969 atggggcgatccaggcctgggtatgagtcagcaaacaccttcttcacagggcagacacctcagcgttagcggagtgatactgg 13048

13049 cttactatgttggcactgatgaggggtgcagtgaaagtgttcatgtggcaggagaaaaaaggctgcaccgggtgcgtcagc 13128

13129 agaatatgtgatacaggatataattccgcttccctcgtctcactgactcgtctacgctcggctcgttcgactgcggcgagcggaa 13208

13209 atggcttacgaacggggcgagatttccctggaagatgccaggaagatacttaacaggggaagtgcagagggcccgcgcaaaag 13288

13289 ccgtttttccataggtcccgccccctgacaagcatcacgaaatctgacgctcaaatcagtggtggcgaaacccgacagg 13368

13369 actataaagataccaggcgtttcccttggcggttccctcgtgcgctctcctgttcttgccttccggtttaccgggtgtcat 13448

13449 tccgctgttatggcgcgtttgtctcattccacgcctgacactcagttccgggttaggcagttcgttccaagctggactgt 13528

13529 atgcacgaaccccccttcagtcgcacgcctgcgccttatccggtaactatcgtcttgagtccaacccggaaagacatgc 13608

13609 aaaagcaccactggcagcagccactggtaattgatttagaggagttagttcgaagtcatgcgcgggttaaggctaaact 13688

13689 gaaaggacaagttttgggtgactgcgctcctccaagccagttacctcgggtcaaaagagttggttagctcagagaaccttcga 13768

13769 aaaaccgccccctgaaggcggttttttcggttttcagagcaagagattacgcgcgagacaaaacgatctcaagaagatcctc 13848

13849 ttattaaggggtctgacgctcagtggaacgaaaactcacgttaagggtatttgggtcatgagattatcaaaaaggatcttc 13928

13929 acctagatccttttaaatataaaatgaagttttaaatcaatctaaagtatatatgagtaaaacttgggtctgacagttacca 14008

14009 atgcttaaatcagtgaggcaccctatctcagcgatctgtctatttcggttcacatagttgcctgactccccgctcgtgtaga 14088

14089 taactacgatacgggagggcttaccatctggccccagtgctgcaatgataccgcgagacccacgctcaccgggtccagat 14168

14169 ttatcagcaataaaccagccagccgggaagggccgagcgcagaagtgggtcctgcaactttatccgctccatccagttctat 14248

14249 taattgttgccgggaagctagagtaagtagttcgcaggttaatatgttgcgcaacggtgttgccattgctgcaggcatcg 14328

14329 tgggtgcacgctcgtcgtttgggtatgggttcattcagctccgggttcccaacgatcaaggcgagttacatgatcccccatg 14408

14409 ctgtcgaaaaaagcggttagcttccctcgggtcctccgatcgttgtcagaagtaagttggccgcagtggttatcactcatggt 14488

14489 tatggcagcactgcataattctcttactgtcatgccatccgtaagatgcttttctgtgactgggtgagtactcaaccaagt 14568

14569 cattctgagaatagtgtatgcggcgacaggtgtgctcttgcggcgctcaacacgggataataaccgcgccacatagcaga 14648

14649 actttaaaagtgtctcatcttggaacagttcttccggggcgaaaactctcaaggatcttaccgctgttgagatccagttc 14728

14729 gatgtaaccactcgtgcacccaactgatcttcagcatcttttactttcaccagcgtttctgggtgagcaaaaaacaggaa 14808

14809 ggcataatgcccgaataaagggaataaggcgacacgggaatgtgaatactcatactcttcttttcaatattattga 14888

14889 agcattttatcagggttattgtctcatgagcggatataatttgaatgtatttagaaaaataacaaataggggttccgcg 14968

14969 cacattttcccgaaaagtgcacacctgacgtcgacctgaggttaattataacccgggccccatataggatccaattctaga 15048

15049 taatacgaactcactata 15065

FIGURE 10-8

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## BVDV NADL (inf. clone) -&gt; Genes

DNA sequence 12578 b.p. gtatacagagaat ... ctaacagccccc linear

1 gtatacagagaattagaaaaggcactcgtatagctattgggcaactaaaaataataattaggcctaggggaacaaatccctc 80  
81 tcagcggaaggccgaaaagaggctagccatgcccttagtaggactagcataatgaggggggtagcaacagtggtgagttcg 160  
161 ttggatggcttaagccctgagtacagggtagtcgtcagtggttcgacgccttggaataaagggtctcgagatgccacgtgg 240  
241 acgagggcatgcccaagcacatcttaacctgagcgggggtcgccaggtaaaagcagttttaaccgactgttacgaata 320  
321 cagcctgatagggtgctgcagagggccactgtattgctactaaaaatctctgtgtacatggcac ATG GAG TTG 394  
1 M E L 3  
395 ATC ACA AAT GAA CTT TTA TAC AAA ACA TAC AAA CAA AAA CCC GTC GGG GTG GAG GAA CCT 454  
4 I T N E L L Y K T Y K Q K P V G V E E P 23  
455 GTT TAT GAT CAG GCA GGT GAT CCC TTA TTT GGT GAA AGG GGA GCA GTC CAC CCT CAA TCG 514  
24 V Y D Q A G D P L F G E R G A V H P Q S 43  
515 ACG CTA AAG CTC CCA CAC AAG AGA GGG GAA CGC GAT GTT CCA ACC AAC TTG GCA TCC TTA 574  
44 T L K L P H K R G E R D V P T N L A S L 63  
575 CCA AAA AGA GGT GAC TGC AGG TCG GGT AAT AGC AGA GGA CCT GTG AGC GGG ATC TAC CTG 634  
64 P K R G D C R S G N S R G P V S G I Y L 83  
635 AAG CCA GGG CCA CTA TTT TAC CAG GAC TAT AAA GGT CCC GTC TAT CAC AGG GCC CCG CTG 694  
84 K P G P L F Y Q D Y K G P V Y H R A P L 103  
695 GAG CTC TTT GAG GAG GGA TCC ATG TGT GAA ACG ACT AAA CGG ATA GGG AGA GTA ACT GGA 754  
104 E L F E E G S M C E T T K R I G R V T G 123  
755 AGT GAC GGA AAG CTG TAC CAC ATT TAT GTG TGT ATA GAT GGA TGT ATA ATA AAA AGT 814  
124 S D G K L Y H I Y V C I D G C I I I K S 143  
815 GCC ACG AGA AGT TAC CAA AGG GTG TTC AGG TGG GTC CAT AAT AGG CTT GAC TGC CCT CTA 874  
144 A T R S Y Q R V F G W V H N R L D C P L 163  
875 TGG GTC ACA ACT TGC TCA GAC ACG AAA GAA GAG GGA ACA AAA AAG AAA ACA CAG AAA 934  
164 W V T T C S D T K E E G A T K K K T Q K 183  
935 CCC GAC AGA CTA GAA AGG GGG AAA ATG AAA ATA GTG CCC AAA GAA TCT GAA AAA GAC AGC 994  
184 P D R L E R G K M K I V P K E S E K D S 203  
995 AAA ACT AAA CCT CCG GAT GCT ACA ATA GTG GTG GAA GGA GTC AAA TAC CAG GTG AGG AAG 1054  
204 K T K P P D A T I V V E G V K Y Q V R K 223  
1055 AAG GGA AAA ACC AAG AGT AAA AAC ACT CAG GAC GGC TTG TAC CAT AAC AAA AAC AAA CCT 1114  
224 K G K T K S N T Q D G L Y H N K N K P 243  
1115 CAG GAA TCA CGC AAG AAA CTG GAA AAA GCA TTG TTG GCG TGG GCA ATA ATA GCT ATA GTT 1174  
244 Q E S R K K L E K A L L A W A I I A I V 263  
1175 TTG TTT CAA GTT ACA ATG GGA GAA AAC ATA ACA CAG TGG AAC CTA CAA GAT AAT GGG ACG 1234  
264 L F Q V T M G E N I T Q W N L Q D N G T 283  
1235 GAA GGG ATA CAA CGG GCA ATG TTC CAA AGG GGT GTG AAT AGA AGT TTA CAT GGA ATC TGG 1294  
284 E G I Q R A M F Q R G V N R S L H G I W 303  
1295 CCA GAG AAA ATC TGT ACT GGT GTC CCT TCC CAT CTA GCC ACC GAT ATA GAA CTA AAA ACA 1354  
304 P E K I C T G V P S H L A T D I E L K T 323  
1355 ATT CAT GGT ATG ATG GAT GCA AGT GAG AAG ACC AAC TAC ACG TGT TGC AGA CTT CAA CGC 1414  
324 I H G M M D A S E K T N Y T C C R L Q R 343  
1415 CAT GAG TGG AAC AAG CAT GGT TGG TGC AAC TGG TAC AAT ATT GAA CCC TGG ATT CTA GTC 1474  
344 H E W N K H G W C N W Y N I E P W I L V 363  
1475 ATG AAT AGA ACC CAA GCC AAT CTC ACT GAG GGA CAA CCA CCA AGG GAG TGC GCA GTC ACT 1534  
364 M N R T Q A N L T E G G P P R E C A V T 383  
1535 TGT AGG TAT GAT AGG GCT AGT GAC TTA AAC GTG GTA ACA CAA GCT AGA GAT AGC CCC ACA 1594  
384 C R Y D R A S D L N V V T Q A R D S P T 403  
1595 CCC TTA ACA GGT TGC AAG AAA GGA AAG AAC TTC TCC TTT GCA GGC ATA TTG ATG CGG GGC 1654  
404 P L T G C K K G K N F S F A G I L M R G 423  
1655 CCC TGC AAC TTT GAA ATA GCT GCA AGT GAT GTA TTA TTC AAA GAA CAT GAA CGC ATT AGT 1714  
424 P C N F E I A A S D V L F K E H E R I S 443  
1715 ATG TTC CAG GAT ACT ACT CTT TAC CTT GTT GAC GGG TTG ACC AAC TCC TTA GAA GGT GCC 1774  
444 M F Q D T T L Y L V D G L T N S L E G A 463

FIGURE 11-1

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BVDV NADL (inf. clone) -&gt; Gen...

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1775 AGA CAA GGA ACC GCT AAA CTG ACA ACC TGG TTA GGC AAG CAG CTC GGG ATA CTA GGA AAA 1834  
464 R Q G T A K L T T W L G K Q L G I L G K 483

1835 AAG TTG GAA AAC AAG AGT AAG ACG TGG TTT GGA GCA TAC GCT GCT TCC CCT TAC TGT GAT 1894  
484 K L E N K S K T W F G A Y A A S P Y C D 503

1895 GTC GAT CGC AAA ATT GGC TAC ATA TGG TAT ACA AAA AAT TGC ACC CCT GCC TGC TTA CCC 1954  
504 V D R K I G Y I W Y T K N C T P A C L P 523

1955 AAG AAC ACA AAA ATT GTC GGC CCT GGG AAA TTT GAC ACC AAT GCA GAG GAC GGC AAG ATA 2014  
524 K N T K I V G P G K F D T N A E D G K I 543

2015 TTA CAT GAG ATG GGG GGT CAC TTG TCG GAG GTA CTA CTA CTT TCT TTA GTG GTG CTG TCC 2074  
544 L H E M G G H L S E V L L L S L V V L S 563

2075 GAC TTC GCA CCG GAA ACA GCT AGT GTA ATG TAC CTA ATC CTA CAT TTT TCC ATC CCA CAA 2134  
564 D F A P E T A S V L I L H F S I P Q 583

2135 AGT CAC GTT GAT GTA ATG GAT TGT GAT AAG ACC CAG TTG AAC CTC ACA GTG GAG CTG ACA 2194  
584 S H V D V M D C D K T Q L N L T V E L T 603

2195 ACA GCT GAA GTA ATA CCA GGG TCG GTC TGG AAT CTA GGC AAA TAT GTA TGT ATA AGA CCA 2254  
604 T A E V I P G S V W N L G K Y V C I R P 623

2255 AAT TGG TGG CCT TAT GAG ACA ACT GTA GTG TTG GCA TTT GAA GAG GTG AGC CAG GTG GTG 2314  
624 N W W P Y E T T V V L A F E E V S Q V V 643

2315 AAG TTA GTG TTG AGG GCA CTC AGA GAT TTA ACA CGC ATT TGG AAC GCT GCA ACA ACT ACT 2374  
644 K L V L R A L R D L T R I W N A A T T T 663

2375 GCT TTT TTA GTA TGC CTT GTT AAG ATA GTC AGG GGC CAG ATG GTA CAG GGC ATT CTG TGG 2434  
664 A F L V C L V K I V R G Q M V Q G I L W 683

2435 CTA CTA TTG ATA ACA GGG GTA CAA GGG CAC TTG GAT TGC AAA CCT GAA TTC TCG TAT GCC 2494  
684 L L L I T G V Q G H L D C K P E F S Y A 703

2495 ATA GCA AAG GAC GAA AGA ATT GGT CAA CTG GGG GCT GAA GGC CTT ACC ACC ACT TGG AAG 2554  
704 I A K D E R I G Q L G A E G L T T W K 723

2555 GAA TAC TCA CCT GGA ATG AAG CTA GAA GAC ACA ATG GTC ATT GCT TGG TGC GAA GAT GGG 2614  
724 E Y S P G M K L E D T M V I A W C E D G 743

2615 AAG TTA ATG TAC CTC CAA AGA TGC ACG AGA GAA ACC AGG TAT CTC GCA ATC TTG CAT ACA 2674  
744 K L M Y L Q R C T R E T R Y L A I L H T 763

2675 AGA GCC TTG CCG ACC AGT GTG GTA TTC AAA AAA CTC TTT GAT GGG CGA AAG CAA GAG GAT 2734  
764 R A L P T S V V F K K L F D G R K Q E D 783

2735 GTA GTC GAA ATG AAC GAC AAC TTT GAA TTT GGA CTC TGC CCA TGT GAT GCC AAA CCC ATA 2794  
784 V V E M N D N F E F L C P C D A K P I 803

2795 GTA AGA GGG AAG TTC AAT ACA ACG CTG CTG AAC GGA CCG GCC TTC CAG ATG GTA TGC CCC 2854  
804 V R G K F N T T L L N G P A F Q M V C P 823

2855 ATA GGA TGG ACA GGG ACT GTA AGC TGT ACG TCA TTC AAT ATG GAC ACC TTA GCC ACA ACT 2914  
824 I G W T G T V S C T S F N M D T L A T T 843

2915 GTG GTA CCG ACA TAT AGA AGG TCT AAA CCA TTC CCT CAT AGG CAA GGC TGT ATC ACC CAA 2974  
844 V V R T Y R R S K P F P H R Q G C I T Q 863

2975 AAG AAT CTG GGG GAG GAT CTC CAT AAC TGC ATC CTT GGA GGA AAT TGG ACT TGT GTG CCT 3034  
864 K N L G E D L H N C I L G G N W T C V P 883

3035 GGA GAC CAA CTA CTA TAC AAA GGG GGC TCT ATT GAA TCT TGC AAG TGG TGT GGC TAT CAA 3094  
884 G D Q L L Y K G G S I E S C K W C G Y Q 903

3095 TTT AAA GAG AGT GAG GGA CTA CCA CAC TAC CCC ATT GGC AAG TGT AAA TTG GAG AAC GAG 3154  
904 F K E S E G L P H Y P I G K C K L E N E 923

3155 ACT GGT TAC AGG CTA GTA GAC AGT ACC TCT TGC AAT AGA GAA GGT GTG GCC ATA GTA CCA 3214  
924 T G Y R L V D S T G C N R E G V A I V P 943

3215 CAA GGG ACA TTA AAG TGC AAG ATA GGA AAA ACA ACT GTA CAG GTC ATA GCT ATG GAT ACC 3274  
944 Q G T L K C K I G K T T V Q V I A M D T 963

3275 AAA CTC GGA CCT ATG CCT TGC AGA CCA TAT GAA ATC ATA TCA AGT GAG GGG CCT GTA GAA 3334  
964 K L G P M P C R P Y E I I S S E G P V E 983

3335 AAG ACA GCG TGT ACT TTC AAC TAC ACT AAG ACA TTA AAA AAT AAG TAT TTT GAG CCC AGA 3394  
984 K T A C T F N Y T K T L K N K Y F E P R 1003

3395 GAC AGC TAC TTT CAG CAA TAC ATG CTA AAA GGA GAG TAT CAA TAC TGG TTT GAC CTG GAG 3454  
1004 D S Y F Q Y M L K G E Y Q Y W F D L E 1023

3455 GTG ACT GAC CAT CAC CGG GAT TAC TTC GCT GAG TCC ATA TTA GTG GTG GTA GTA GCC CTC 3514  
1024 V T D H H R D Y F A E S I L V V V V A L 1043

FIGURE 11-2

BVDV NADL (inf. clone) -&gt; Ge...

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3515 TTG GGT GGC AGA TAT GTA CTT TGG TTA CTG GTT ACA TAC ATG GTC TTA TCA GAA CAG AAG 3574  
1044 L G G R Y V L W L L V T Y M V L S E Q K 1063

3575 GCC TTA GGG ATT CAG TAT GGA TCA GGG GAA GTG GTG ATG ATG GGC AAC TTG CTA ACC CAT 3634  
1064 A L G I Q Y C S G E V V M M G N L L T H 1083

3635 AAC AAT ATT GAA GTG CTG ACA TAC TTC TTG CTG CTG TAC CTA CTG CTG AGG GAG GAG AGC 3694  
1084 N N I E V V T Y F L L Y L L R E E S 1103

3695 GTA AAG AAG TGG GTC TTA CTC TTA TAC CAC ATC TTA GTG GTA CAC CCA ATC AAA TCT GTA 3754  
1104 V K K W V L L L Y H I L V V H P I K S V 1123

3755 ATT GTG ATC CTA CTG ATG ATT GGG GAT GTG GTA AAG GCC GAT TCA GGG GGC CAA GAG TAC 3814  
1124 I V I L L M I G D V V K A D S G G Q E Y 1143

3815 TTG GGG AAA ATA GAC CTC TGT TTT ACA GTA GTA CTA ATC GTC ATA GGT TTA ATC ATA 3874  
1144 L G K I T C F L V V L I V I G L I I 1163

3875 GCC AGG CGT GAC CCA ACT ATA GTG CCA CTG GTA ACA ATA ATG GCA GCA CTG AGG GTC ACT 3934  
1164 A R R D P T I V P L V T I M A A L R V T 1183

3935 GAA CTG ACC CAC CAG CCT GGA GTT GAC ATC GCT GTG GCG GTC ATG ACT ATA ACC CTA CTG 3994  
1184 E L T H Q P G V D I A V A V M T I T L L 1203

3995 ATG GTT AGC TAT GTG ACA GAT TAT TTT AGA TAT AAA AAA TGG TTA CAG TGC ATT CTC AGC 4054  
1204 M V S Y V T D Y F R Y K K W L Q C I L S 1223

4055 CTG GTA TCT GCG GTG TTC TTG ATA AGA AGC CTA ATA TAC CTA GGT AGA ATC GAG ATG CCA 4114  
1224 L V S A V F L I R S L I Y L G R I E M P 1243

4115 GAG GTA ACT ATC CCA AAC TGG AGA CCA CTA ACT TTA ATA CTA TTA TAT TTG ATC TCA ACA 4174  
1244 E V T I P N W R P L T L I L L Y L I S T 1263

4175 ACA ATT GTA ACG AGG TGG AAG GTT GAC GTG GCT GGC CTA TTG TTG CAA TGT GTG CCT ATC 4234  
1264 T I V T R W K V D V A G L L L Q C V P I 1283

4235 TTA TTG CTG GTC ACA ACC TTG TGG GCC GAC TTC TTA ACC CTA ATA CTG ATC CTG CCT ACC 4294  
1284 L L L V T T L W A D F L T L I L I L P T 1303

4295 TAT GAA TTG GTT AAA TTA TAC TAT CTG AAA ACT GTT AGG ACT GAT ATA GAA AGA AGT TGG 4354  
1304 Y E L V K L Y Y L K T V R T D I E R S W 1323

4355 CTA GGG GGG ATA GAC TAT ACA AGA GTT GAC TCC ATC TAC GAC GTT GAT GAG AGT GGA GAG 4414  
1324 L G G I D Y T R V D S I Y D V D E S G E 1343

4415 GGC GTA TAT CTT TTT CCA TCA AGG CAG AAA GCA CAG GGG AAT TTT TCT ATA CTC TTG CCC 4474  
1344 G V Y L F P S R Q K A Q G N F S I L L P 1363

4475 CTT ATC AAA GCA ACA CTG ATA AGT TGC GTC AGC AGT AAA TGG CAG CTA ATA TAC ATG AGT 4534  
1364 L I K A T I S C V S S K W C L I Y M S 1383

4535 TAC TTA ACT TTG GAC TTT ATG TAC TAC ATG CAC AGG AAA GTT ATA GAA GAG ATC TCA GGA 4594  
1384 Y L T L D F M Y Y M H R K V I E E I S G 1403

4595 GGT ACC AAC ATA ATA TCC AGG TTA GTG GCA GCA CTC ATA GAG CTG AAC TGG TCC ATG GAA 4654  
1404 G T N I I S R L V A A L I E L N W S M E 1423

4655 GAA GAG GAG AGC AAA GGC TTA AAG AAG TTT TAT CTA TTG TCT GGA AGG TTG AGA AAC CTA 4714  
1424 E E S K G L K K F Y L L S G R L R N L 1443

4715 ATA ATA AAA CAT AAG GTA AGG AAT GAG ACC GTG GCT TCT TGG TAC GGG GAG GAG GAA GTC 4774  
1444 I I K H K V R N E T V A S W Y G E E V 1463

4775 TAC GGT ATG CCA AAG ATC ATG ACT ATA ATC AAG GCC AGT ACA CTG AGT AAG AGC AGG CAC 4834  
1464 Y G M P K I M T I I K A S T L S K S R H 1483

4835 TGC ATA ATA TGC ACT GTA TGT GAG GGC CGA GAG TGG AAA GGT GGC ACC TGC CCA AAA TGT 4894  
1484 C I I C T V C E G R E W K G G T C P K C 1503

4895 GGA CGC CAT GGG AAG CCG ATA ACG TGT GGG ATG TCG CTA GCA GAT TTT GAA GAA AGA CAC 4954  
1504 G R H G K P I T C G M S L A D F E E R H 1523

4955 TAT AAA AGA ATC TTT ATA AGG GAA GGC AAC TTT GAG GGT ATG TGC AGC CGA TGC CAG GGA 5014  
1524 Y K R I F I R E G N F E G M C S R C Q G 1543

5015 AAG CAT AGG AGG TTT GAA ATG GAC CGG GAA CCT AAG AGT GCC AGA TAC TGT GCT GAG TGT 5074  
1544 K H R R F E M D R E P K S A R Y C A E C 1563

5075 AAT AGG CTG CAT CCT GCT GAG GAA GGT GAC TTT TGG GCA GAG TCG AGC ATG TTG GGC CTC 5134  
1564 N R L H P A E G A D F V A E S S M L G L 1583

5135 AAA ATC ACC TAC TTT GCG CTG ATG GAT GGA AAG GTG TAT GAT ATC ACA GAG TGG GCT GGA 5194  
1584 K I T Y F A L M D G K V Y D I T E W A G 1603

5195 TGC CAG CGT GTG GGA ATC TCC CCA GAT ACC CAC AGA GTC CCT TGT CAC ATC TCA TTT GGT 5254  
1604 C Q R V G I S P D T H R V P C H I S F G 1623

FIGURE 11-3

BVDV NADL (inf. clone) -&gt; G.

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5255 TCA CGG ATG CCT TTC AGG CAG GAA TAC AAT GGC TTT GTA CAA TAT ACC GCT AGG GGG CAA 5314  
1624 S R M P F R Q E Y N G F V Q Y T A R G Q 1643

5315 CTA TTT CTG AGA AAC TTG CCC GTA CTG GCA ACT AAA GTA AAA ATG CTC ATG GTA GGC AAC 5374  
1644 L F L R N L P V L A T K V K M L M V G N 1663

5375 CTT GGA GAA GAA ATT GGT AAT CTG GAA CAT CTT GGG TGG ATC CTA AGG GGG CCT GCC GTG 5434  
1664 L G E E I G N L E H L G W I L R G P A V 1683

5435 TGT AAG AAG ATC ACA GAG CAC GAA AAA TGC CAC ATT AAT ATA CTG GAT AAA CTA ACC GCA 5494  
1684 C K K I T E H E K C H I N I L D K L T A 1703

5495 TTT TTC GGG ATC ATG CCA AGG GGG ACT ACA CCC AGA GCC CCG GTG AGG TTC CCT ACG AGC 5554  
1704 F F G I M P R G T T P R A P V R F P T S 1723

5555 TTA CTA AAA GTG AGG AGG GGT CTG GAG ACT GCC TGG GCT TAC ACA CAC CAA GGC GGG ATA 5614  
1724 L L K V R R G L E T A W A Y T H Q G G I 1743

5615 AGT TCA GTC GAC CAT GTA ACC GCC GGA AAA GAT CTA CTG GTC TGT GAC AGC ATG GGA CGA 5674  
1744 S S V D H V T A G K D L L V C D S M G R 1763

5675 ACT AGA GTG GTT TGC CAA AGC AAC AAC AGG TTG ACC GAT GAG ACA GAG TAT GGC GTC AAG 5734  
1764 T R V V C Q S N N R L T D E T E Y G V K 1783

5735 ACT GAC TCA GGG TGC CCA GAC GGT GCC AGA CAG TGT TAT GTG TTA AAT CCA GAG GCC GTT AAC 5794  
1784 T D S G C P D G A R C Y V L N P E A V N 1803

5795 ATA TCA GGA TCC AAA GGG GCA GTC GTT CAC CTC CAA AAG ACA GGT GGA GAA TTC ACG TGT 5854  
1804 I S G S K G A V V H L Q K T G G E F T C 1823

5855 GTC ACC GCA TCA GGC ACA CCG GCT TTC TTC GAC CTA AAA AAC TTG AAA GGA TGG TCA GGC 5914  
1824 V T A S G T P A F F D L K N L K G W S G 1843

5915 TTG CCT ATA TTT GAA GCC TCC AGC GGG AGG GTG GTT GGC AGA GTC AAA GTA GGG AAG AAT 5974  
1844 L P I F E A S S G R V V G R V K V G K N 1863

5975 GAA GAG TCT AAA CCT ACA AAA ATA ATG AGT GGA ATC CAG ACC GTC TCA AAA AAC AGA GCA 6034  
1864 E E S K P T K I M S G I Q T V S K N R A 1883

6035 GAC CTG ACC GAG ATG GTC AAG AAG ATA ACC AGC ATG AAC AGG GGA GAC TTC AAG CAG ATT 6094  
1884 D L T E M V K K I T S M N R G D F K Q I 1903

6095 ACT TTG GCA ACA GGG GCA GGC AAA ACC ACA GAA CTC CCA AAA GCA GTT ATA GAG GAG ATA 6154  
1904 T L A T G A G K T T E L P K A V I E E I 1923

6155 GGA AGA CAC AAG AGA GTA TTA GTT CTT ATA CCA TTA AGG GCA GCG GCA GAG TCA GTC TAC 6214  
1924 G R H K R V L V L I P L R A A A E S V Y 1943

6215 CAG TAT ATG AGA TTG AAA CAC CCA AGC ATC TCT TTT AAC CTA AGG ATA GGG GAC ATG AAA 6274  
1944 Q Y M R L K H P S I S F N L R I G D M K 1963

6275 GAG GGG GAC ATG GCA ACC GGG ATA ACC TAT GCA TCA TAC GGG TAC TTC TGC CAA ATG CCT 6334  
1964 E G D M A T G I T Y A S Y G Y F C Q M P 1983

6335 CAA CCA AAG CTC AGA GCT GCT ATG GTA GAA TAC TCA TAC ATA TTC TTA GAT GAA TAC CAT 6394  
1984 Q P K L R A A M V E Y S Y I F L D E Y H 2003

6395 TGT GCC ACT CCT GAA CAA CTG GCA ATT ATC GGG AAG ATC CAC AGA TTT TCA GAG AGT ATA 6454  
2004 C A T P E Q L A I I G K I H R F S E S I 2023

6455 AGG GTT GTC GCC ATG ACT GCC ACG CCA GCA GGG TCG GTG ACC ACA ACA GGT CAA AAG CAC 6514  
2024 R V V A M T A T P A G S V T T T G Q K H 2043

6515 CCA ATA GAG GAA TTC ATA GCC CCC GAG GTA ATG AAA GGG GAG GAT CTT GGT AGT CAG TTC 6574  
2044 P I E E F I A P E V M K G E D L G S Q F 2063

6575 CTT GAT ATA GCA GGG TTA AAA ATA CCA GTG GAT GAG ATG AAA GGC AAT ATG TTG GTT TTT 6634  
2064 L D I A G L K I P V D E M K G N M L V F 2083

6635 GTA CCA ACG AGA AAC ATG GCA GTA GAG GTA CCA AAG AAG CTA AAA GCT AAG GGC TAT AAC 6694  
2084 V P T R N M A V E V A K K L K A K G Y N 2103

6695 TCT GGA TAC TAT TAC AGT GGA GAG GAT CCA GCC AAT CTG AGA GTT GTG ACA TCA CAA TCC 6754  
2104 S G Y Y Y S G E D P A N L R V V T S Q S 2123

6755 CCC TAT GTA ATC GTG GCT ACA AAT GCT ATT GAA TCA GGA GTG ACA CTA CCA GAT TTG GAC 6814  
2124 P Y V I V A T N A I E S G V T L P D L D 2143

6815 ACG GTT ATA GAC ACG GGG TTG AAA TGT GAA AAG AGG GTG AGG GTA TCA TCA AAG ATA CCC 6874  
2144 T V I D T G L K C E K R V R V S S K I P 2163

6875 TTC ATC GTA ACA GGC CTT AAG AGG ATG GCC GTG ACT GTG GGT GAG CAG GCG CAG CGT AGG 6934  
2164 F I V T G L K R M A V T V G E Q A Q R R 2183

6935 GGC AGA GTA GGT AGA GTG AAA CCC GGG AGG TAT TAT AGG AGC CAG GAA ACA GCA ACA GGG 6994  
2184 G R V G R V K P G R Y Y R S Q E T A T G 2203

FIGURE 11-4

BVDV NADL (inf. clone) -&gt; Gc

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6995 TCA AAG GAC TAC CAC TAT GAC CTC TTG CAG GCA CAA AGA TAC GGG ATT GAG GAT GGA ATC 7054  
2204 S K D Y H Y D L L Q A Q R Y G I E D G I 2223

7055 AAC GTG ACG AAA TCC TTT AGG GAG ATG AAT TAC GAT TGG AGC CTA TAC GAG GAG GAC AGC 7114  
2224 N V T K S F R E M N Y D W S L Y E E D S 2243

7115 CTA CTA ATA ACC CAG CTG GAA ATA CTA AAT AAT CTA CTC ATC TCA GAA GAC TTG CCA GCC 7174  
2244 L L I T Q L E I L N N L L I S E D L P A 2263

7175 GCT GTT AAG AAC ATA ATG GCC AGG ACT GAT CAC CCA GAG CCA ATC CAA CTT GCA TAC AAC 7234  
2264 A V K N I M A R T D H P E P I Q L A Y N 2283

7235 AGC TAT GAA GTC CAG GTC CCG GTC CTG TTC CCA AAA ATA AGG AAT GGA GAA GTC ACA GAC 7294  
2284 S Y E V Q V P L F P K I R N G E V T D 2303

7295 ACC TAC GAA AAT TAC TCG TTT CTA AAT GCC AGA AAG TTA GGG GAG GAT GTG CCC GTG TAT 7354  
2304 T Y E H Y S F L N A R K L G E D V P V Y 2323

7355 ATC TAC GCT ACT GAA GAT GAG GAT CTG GCA GTT GAC CTC TTA GGG CTA GAC TGG CCT GAT 7414  
2324 I Y A T E D E D L A V D L L G L D W P D 2343

7415 CCT GGG AAC CAG CAG GTA GTG GAG ACT GGT AAA GCA CTG AAG CAA GTG ACC GGG TTG TCC 7474  
2344 P G N Q Q V V E T G K A L K Q V T G L S 2363

7475 TCG GCT GAA AAT GCC CTA CTA GTG GCT TTA TTT GGG TAT GTG GGT TAC CAG GCT CTC TCA 7534  
2364 S A E N A L V A L F G Y V G Y Q A L S 2383

7535 AAG AGG CAT GTC CCA ATG ATA ACA GAC ATA TAT ACC ATC GAG GAC CAG AGA CTA GAA GAC 7594  
2384 K R H V P M I T D I Y T I E D Q R L E D 2403

7595 ACC ACC CAC CTC CAG TAT GCA CCC AAC GCC ATA AAA ACC GAT GGG ACA GAG ACT GAA CTG 7654  
2404 T T H L Q Y A P N A I K T D G T E T E L 2423

7655 AAA GAA CTG GCG TCG GGT GAC GTG GAA AAA ATC ATG GGA GCC ATT TCA GAT TAT GCA GCT 7714  
2424 K E L A S G D V E K I M G A I S D Y A A 2443

7715 GGG GAG CTG GAG TTT GTT AAA TCC CAA GCA GAA AAG ATA AAA ACA GCT CCT TTG TTT AAA 7774  
2444 G G L E F V K S Q A E K I K T A P L F K 2463

7775 GAA AAC GCA GAA GCC GCA AAA GGG TAT GTC CAA AAA TTC ATT GAC TCA TTA ATT GAA AAT 7834  
2464 E N A E A A K G Y V Q K F I D S L I E N 2483

7835 AAA GAA GAA ATA ATC AGA TAT GGT TTG TGG GGA ACA CAC ACA GCA CTA TAC AAA AGC ATA 7894  
2484 K E E I I R Y G L W G T H T A L Y K S I 2503

7895 GCT GCA AGA CTG GGG CAT GAA ACA GCG TTT GCC ACA CTA GTG TTA AAG TGG CTA GCT TTT 7954  
2504 A A R L G H E T A F C L V L K W L A F 2523

7955 GGA GGG GAA TCA GTG TCA GAC CAC GTC AAG CAG GCG GCA GTT GAT TTA GTG GTC TAT TAT 8014  
2524 G G E S V S D H V K Q A A V D L V V Y Y 2543

8015 GTG ATG AAT AAG CCT TCC TTC CCA GGT GAC TCC GAG ACA CAG CAA GAA GGG AGG CGA TTC 8074  
2544 V M N K P S F P G D S E T Q Q E G R R F 2563

8075 GTC GCA AGC CTG TTC ATC TCC GCA CTG GCA ACC TAC ACA TAC AAA ACT TGG AAT TAC CAC 8134  
2564 V A S L F I S A L A T Y T Y K T W N Y H 2583

8135 AAT CTC TCT AAA GTG GTG GAA CCA GCC CTG GCT TAC CTC CCC TAT GCT ACC AGC GCA TTA 8194  
2584 N L S K A V P A L A Y L P Y A T S A L 2603

8195 AAA ATG TTC ACC CCA ACG CCG CTG GAG AGC GTG GTG ATA CTG AGC ACC ACG ATA TAT AAA 8254  
2604 K M F T P T R L E S V V I L S T T I Y K 2623

8255 ACA TAC CTC TCT ATA AGG AAG GGG AAG AGT GAT GGA TTG CTG GGT ACG GGG ATA AGT GCA 8314  
2624 T Y L S I R K G K S D G L L G T G I S A 2643

8315 GCC ATG GAA ATC CTG TCA CAA AAC CCA GTA TCG GTA GGT ATA TCT GTG ATG TTG GGG GTA 8374  
2644 A M E I L S Q N P V S V G I S V M L G V 2663

8375 GGG GCA ATC GCT GCG CAC AAC GCT ATT GAG TCC AGT GAA CAG AAA AGG ACC CTA CTT ATG 8434  
2664 G A I A A H N A I E S S E Q K R T L L M 2683

8435 AAG GTG TTT GTA AAG AAC TTC TTG GAT CAG GCT GCA ACA GAT GAG CTG GTA AAA GAA AAC 8494  
2684 K V F V K N F L D Q A A T D E L V K E N 2703

8495 CCA GAA AAA ATT ATA ATG GCC TTA TTT GAA GCA GTC CAG ACA ATT GGT AAC CCC CTG AGA 8554  
2704 P E K I I M A L F E A V Q T I G N P L R 2723

8555 CTA ATA TAC CAC CTG TAT GGG GTT TAC TAC AAA GGT TGG GAG GCC AAG GAA CTA TCT GAG 8614  
2724 L I Y H L Y G V Y Y K W E A K E L S E 2743

8615 AGG ACA GCA GGC AGA AAC TTA TTC ACA TTG ATA ATG TTT GAA GCC TTC GAG TTA TTA GGG 8674  
2744 R T A G R N L F T L I M F E A F E L L G 2763

8675 ATG GAC TCA CAA GGG AAA ATA AGG AAC CTG TCC GGA AAT TAC ATT TTG GAT TTG ATA TAC 8734  
2764 M D S Q G K I R N L S G N Y I L D L I Y 2783

FIGURE 11-5

BVDV NADL (inf. clone) -> G<sub>1</sub> 27/67 4/21/99 5:42:22 PM Page 6

8735 GGC CTA CAC AAG CAA ATC AAC AGA GGG CTG AAG AAA ATG GTA CTG GGG TGG GCC CCT GCA 8794  
2784 G L H K Q I N R G L K K M V L G W A P A 2803

8795 CCC TTT AGT TGT GAC TGG ACC CCT AGT GAC GAG AGG ATC AGA TTG CCA ACA GAC AAC TAT 8854  
2804 P F S C D W T P S D E R I R L P T D N Y 2823

8855 TTG AGG GTA GAA ACC AGG TGC CCA TGT GGC TAT GAG ATG AAA GCT TTC AAA AAT GTA GGT 8914  
2824 L R V E T R C P C G Y E M K A F K N V G 2843

8915 GGC AAA CTT ACC AAA GTG GAG GAG AGC GGG CCT TTC CTA TGT AGA AAC AGA CCT GGT AGG 8974  
2844 G K L T K V E E S G P F L C R N R P G R 2863

8975 GGA CCA GTC AAC TAC AGA GTC ACC AAG TAT TAC GAT GAC AAC CTC AGA GAG ATA AAA CCA 9034  
2864 G P V N Y R V T K Y Y D D N L R E I K P 2883

9035 GTA GCA AAG TTG GAA GGA CAG GTA GAG CAC TAC TAC AAA GGG GTC ACA GCA AAA ATT GAC 9094  
2884 V A K L E G Q V E H Y K G V T A K I D 2903

9095 TAC AGT AAA GGA AAA ATG CTC TTG GCC ACT GAC AAG TGG GAG GTG GAA CAT GGT GTC ATA 9154  
2904 Y S K G K M L L A T D K W E V E H G V I 2923

9155 ACC AGG TTA GCT AAG AGA TAT ACT GGG GTC GGG TTC AAT GGT GCA TAC TTA GGT GAC GAG 9214  
2924 T R L A K R Y T G V G F N G A Y L G D E 2943

9215 CCC AAT CAC CGT GCT CTA GTG GAG AGG GAC TGT GCA ACT ATA ACC AAA AAC ACA GTA CAG 9274  
2944 P N H R A L V E R D C A T I T K N T V Q 2963

9275 TTT CTA AAA ATG AAG AAG GGG TGT GCG TTC ACC TAT GAC CTG ACC ATC TCC AAT CTG ACC 9334  
2964 F L K M K K G C A T Y D L T I S N L T 2983

9335 AGG CTC ATC GAA CTA GTA CAC AGG AAC AAT CTT GAA GAG AAG GAA ATA CCC ACC GCT ACG 9394  
2984 R L I E L V H R N N L E E K E I P T A T 3003

9395 GTC ACC ACA TGG CTA GCT TAC ACC TTC GTG AAT GAA GAC GTA GGG ACT ATA AAA CCA GTA 9454  
3004 V T T W L A Y T F V N E D V G T I K P V 3023

9455 CTA GGA GAG AGA GTA ATC CCC GAC CCT GTA GTT GAT ATC AAT TTA CAA CCA GAG GTG CAA 9514  
3024 L G E R I P D C P V V D I N L Q P E V Q 3043

9515 GTG GAC ACG TCA GAG GTT GGG ATC ACA ATA ATT GGA AGG GAA ACC CTG ATG ACA ACG GGA 9574  
3044 V D T S E V G I T I I G R E T L M T T G 3063

9575 GTG ACA CCT GTC TTG GAA AAA GTA GAG CCT GAC GCC AGC GAC AAC CAA AAC TCG GTG AAG 9634  
3064 V T P V L E K V E P D A S D N Q N S V K 3083

9635 ATC GGG TTG GAT GAG GGT AAT TAC CCA GGG CCT GGA ATA CAG ACA CAT ACA CTA ACA GAA 9694  
3084 I G L D E G N Y P G P G I Q T H T L T E 3103

9695 GAA ATA CAC AAC AGG GAT GCG AGG CCC TTC ATC ATG ATC CTG GGC TCA AGG AAT TCC ATA 9754  
3104 E I H N D A R P F I M I L G S R N S I 3123

9755 TCA AAT AGG GCA AAG ACT GCT AGA AAT ATA AAT CTG TAC ACA GGA AAT GAC CCC AGG GAA 9814  
3124 S N R A K T A R N I N L Y T G N D P R E 3143

9815 ATA CGA GAC TTG ATG GCT GCA GGG CGC ATG TTA GTA GTA GCA CTG AGG GAT GTC GAC CCT 9874  
3144 I R D L M A A G R M L V V A L R D V D P 3163

9875 GAG CTG TCT GAA ATG GTC GAT TTC AAG GGG ACT TTT TTA GAT AGG GAG GCC CTG GAG GCT 9934  
3164 E L S E M V D F K G T F L D R E A L E A 3183

9935 CTA AGT CTC GGG CAA CCT AAA CCG AAG CAG GTT ACC AAG GAA GCT GTT AGG AAT TTG ATA 9994  
3184 L S L G Q P K P K Q V T K E A V R N L I 3203

9995 GAA CAG AAA AAA GAT GTG GAG ATC CCT AAC TGG TTT GCA TCA GAT GAC CCA GTA TTT CTG 10054  
3204 E Q K K D V E I P N W F A S D D P V F L 3223

10055 GAA GTG GCC TTA AAA AAT GAT AAG TAC TAC TTA GTA GGA GAT GTT GGA GAG CTA AAA GAT 10114  
3224 E V A L K N D K Y Y L V G D V G E L K D 3243

10115 CAA GCT AAA GCA CTT GGG GCC ACG GAT CAG ACA AGA ATT ATA AAG GAG GTA GGC TCA AGG 10174  
3244 Q A K A L G A F T D Q T R I I K E V G S R 3263

10175 ACG TAT GCC ATG AAG CTA TCT AGC TGG TTC CTC AAG GCA TCA AAC AAA CAG ATG AGT TTA 10234  
3264 T Y A M K L S S W F L K A S N K Q M S L 3283

10235 ACT CCA CTG TTT GAG GAA TTG TTG CTA CCG TGC CCA CCT GCA ACT AAG AGC AAT AAG GGG 10294  
3284 T P L F E E L L L R C P P A T K S N K G 3303

10295 CAC ATG GCA TCA GCT TAC CAA TTG GCA CAG GGT AAC TGG GAG CCC CTC GGT TGC GGG GTG 10354  
3304 H M A S A Y Q L A A G G N W E P L G C G V 3323

10355 CAC CTA GGT ACA ATA CCA GCC AGA AGG GTG AAG ATA CAC CCA TAT GAA GCT TAC CTG AAG 10414  
3324 H L G T I P A R R V K I H P Y E A Y L K 3343

10415 TTG AAA GAT TTC ATA GAA GAA GAA GAG AAG AAA CCT AGG GTT AAG GAT ACA GTA ATA AGA 10474  
3344 L K D F I E E E E K K P R V K D T V I R 3363

FIGURE 11-6

BVDV NADL (inf. clone) -&gt; G. s

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10475 GAG CAC AAC AAA TGG ATA CTT AAA AAA ATA AGG TTT CAA GGA AAC CTC AAC ACC AAG AAA 10534  
3364 E H N K W I L K K I R F Q G N L N T K K 3383

10535 ATG CTC AAC CCG GGG AAA CTA TCT GAA CAG TTG GAC AGG GAG GGG CGC AAG AGG AAC ATC 10594  
3384 M L N P G K L S E Q L D R E G R K R N I 3403

10595 TAC AAC CAC CAG ATT GGT ACT ATA ATG TCA AGT GCA GGC ATA AGG CTG GAG AAA TTG CCA 10654  
3404 Y N H Q I G T I M S S A G I R L E K L P 3423

10655 ATA GTG AGG GCC CAA ACC GAC ACC AAA ACC TTT CAT GAG GCA ATA AGA GAT AAG ATA GAC 10714  
3424 I V R A Q T D T K T F H E A I R D K I D 3443

10715 AAG AGT GAA AAC CCG CAA AAT CCA GAA TTG CAC AAC AAA TTG TTG GAG ATT TTC CAC ACG 10774  
3444 K S E N R Q N P E L H N K L L E I F H T 3463

10775 ATA GCC CAA CCC ACC CTG AAA CAC ACC TAC GGT GAG GTG ACG TGG GAG CAA CTT GAG CGC 10834  
3464 I A Q P T L K K H T Y G E V T W E Q L E A 3483

10835 GGG ATA AAT AGA AAG GGG GCA GCA GGC TTC CTG GAG AAG AAG AAC ATC GGA GAA GTA TTG 10894  
3484 G I N R K G A A G F L E K K N I G E V L 3503

10895 GAT TCA GAA AAG CAC CTG GTA GAA CAA TTG GTC AGG GAT CTG AAG GCC GGG AGA AAG ATA 10954  
3504 D S E K H L V E Q L V R D L K A G R K I 3523

10955 AAA TAT TAT GAA ACT GCA ATA CCA AAA AAT GAG AAG AGA GAT GTC AGT GAT GAC TGG CAG 11014  
3524 K Y Y E T A I P K N E K R D V S D W Q 3543

11015 GCA GGG GAC CTG GTG GTT GAG AAG AGG CCA AGA GTT ATC CAA TAC CCT GAA GCC AAG ACA 11074  
3544 A G D L V V E K R P R V I Q Y P E A K T 3563

11075 AGG CTA GCC ATC ACT AAG GTC ATG TAT AAC TGG GTG AAA CAG CAG CCC GTT GTG ATT CCA 11134  
3564 R L A I T K V M Y N W V K Q Q P V V I P 3583

11135 GGA TAT GAA GGA AAG ACC CCC TTG TTC AAC ATC TTT GAT AAA GTG AGA AAG GAA TGG GAC 11194  
3584 G Y E G K T P L F N I F D K V R K E W D 3603

11195 TCG TTC AAT GAG CCA GTG GCC GTA AGT TTT GAC ACC AAA GCC TGG GAC ACT CAA GTG ACT 11254  
3604 S F N E P V A V S F D T K A W D T Q V T 3623

11255 AGT AAG GAT CTG CAA CTT ATT GGA GAA ATC CAG AAA TAT TAC TAT AAG AAG GAG TGG CAC 11314  
3624 S K D L Q L I G E I Q K Y Y Y K K E W H 3643

11315 AAG TTC ATT GAC ACC ATC ACC GAC CAC ATG ACA GAA GTA CCA GTT ATA ACA GCA GAT GGT 11374  
3644 K F I D T I T D H M T E V P V I T A D G 3663

11375 GAA GTA TAT ATA AGA AAT GGG CAG AGA GGG AGC GGC CAG CCA GAC ACA AGT GCT GGC AAC 11434  
3664 E V Y I R N G Q R G S G Q P D T S A G N 3683

11435 AGC ATG TTA AAT GTC CTG ACA ATG ATG TAC GGC TTC TGC GAA AGC ACA GGG GTA CCG TAC 11494  
3684 S M L N V L T M M Y C F C E S T G V P Y 3703

11495 AAG AGT TTC AAC AGG GTG GCA AGG ATC CAC GTC TGT GGG GAT GAT GGC TTC TTA ATA ACT 11554  
3704 K S F N R V A R I H V C G D D G F L I T 3723

11555 GAA AAA GGG TTA GGG CTG AAA TTT GCT AAC AAA GGG ATG CAG ATT CTT CAT GAA GCA GGC 11614  
3724 E K G L G L K F A N K G M Q I L H E A G 3743

11615 AAA CCT CAG AAG ATA ACG GAA GGG GAA AAG ATG AAA GTT GCC TAT AGA TTT GAG GAT ATA 11674  
3744 K P Q K I T E G E K M K V A Y R F E D I 3763

11675 GAG TTC TGT TCT CAT ACC CCA GTC CCT GTT AGG TGG TCC GAC AAC ACC AGT AGT CAC ATG 11734  
3764 E F C S H T P V P V R W S D N T S S H M 3783

11735 GCC GGG AGA GAC ACC GCT GTG ATA CTA TCA AAG ATG GCA ACA AGA TTG GAT TCA AGT GGA 11794  
3784 A G R D T A V I L S K M A T R L D S S G 3803

11795 GAG AGG GGT ACC ACA GCA TAT GAA AAA GCG GTA GCC TTC AGT TTC TTG CTG ATG TAT TCC 11854  
3804 E R G T T A Y E K A V A F S F L L M Y S 3823

11855 TGG AAC CCG CTT GTT AGG AGG ATT TGC CTG TTG GTC CTT TCG CAA CAG CCA GAG ACA GAC 11914  
3824 W N P L V R R I C L V L S Q Q P E T D 3843

11915 CCA TCA AAA CAT GCC ACT TAT TAT TAC AAA GGT GAT CCA ATA GGG GCC TAT AAA GAT GTA 11974  
3844 P S K H A T Y Y Y K G D P I G A Y K D V 3863

11975 ATA GGT CCG AAT CTA AGT GAA CTG AAG AGA ACA GGC TTT GAG AAA TTG GCA AAT CTA AAC 12034  
3864 I G R N L S E L K R T G F E K L A N L N 3883

12035 CTA AGC CTG TCC ACG TTG GGG ATC TGG ACT AAG CAC ACA AGC AAA AGA ATA ATT CAG GAC 12094  
3884 L S L S T L G I W T K H T S K R I I Q D 3903

12095 TGT GTT GCC ATT GGG AAA GAA GAG GGC AAC TGG CTA GTT AAC GCC GAC AGG CTG ATA TCC 12154  
3904 C V A I G K E E G N W L V N A D R L I S 3923

12155 AGC AAA ACT GGC CAC TTA TAC ATA CCT GAT AAA GGC TTT ACA TTA CAA GGA AAG CAT TAT 12214  
3924 S K T G H L Y I P D K G F T L Q G K H Y 3943

FIGURE 11-7



BVDV NADL (inf. clone) -&gt; Genes

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12215 GAG CAA CTG CAG CTA AGA ACA GAG ACA AAC CCG GTC ATG GGG GTT GGG ACT GAG AGA TAC 12274
3944 E Q L Q L R T E T N P V M G V G T E R Y 3963

12275 AAG TTA GGT CCC ATA GTC AAT CTG CTG CTG AGA AGG TTG AAA ATT CTG CTC ATG ACG GCC 12334
3964 K L G P I V N L L L R R L K I L L M T A 3983

12335 GTC GGC GTC AGC AGC TGA gacaaaatgtatatattgtaaaataaattaatccatgtacatagtgcatataaatat 12408
3984 V G V S S S 3989

12409 agttgggaccgtccacctcaagaagacgacacgccccaacacgcacagctaaacagtagtcaagattatctacctcaagat 12488

12489 aacactacatttaatgcacacagcactttagctgtatgaggatagccccgacgtctatagttggactaggggaagacctct 12568

12569 aacagcccc 12578
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FIGURE 11-8

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## BVDV NADL cIns- (inf. clone) -&gt; Genes

DNA sequence 12308 b.p. gtatacagagaat ... ctaacagccccc linear

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1  gtatacagagaattagaaaaggcactcgatatacgtattgggcaattaaaaataataattaggcctaggggaacaaatccctc 80
81  tcagcgaaggccgaaaagaggctagccatgcccttagtaggactagcataatgaggggggtagcaacagtggtaggttcg 160
161  ttggatggcttaagccctgagtagcagggtagtcgctcagtggttcgacgccttggaataaagggtctcagatgccacgtgg 240
241  acgaggggcatgccccaaagcacatcttaacctgagcgggggtcgccccaggtaaaagcagttttaaccgactgttacgaata 320
321  cagcctgataggggtgctgcagaggcccactgtattgctactaaaaatctctgctgtacatggcac ATG GAG TTG 394
1      M   E   L   3
395  ATC ACA AAT GAA CTT TTA TAC AAA ACA TAC AAA CAA AAA CCC GTC GGG GTG GAG GAA CCT 454
4      I   T   N   E   L   L   Y   K   T   Y   K   Q   K   P   V   G   V   E   E   P   23
455  GTT TAT GAT CAG GCA GGT GAT CCC TTA TTT GGT GAA AGG GGA GCA GTC CAC CCT CAA TCG 514
24      V   Y   D   Q   A   G   D   P   L   F   G   E   R   G   A   V   H   P   Q   S   43
515  ACG CTA AAG CTC CCA CAC AAG AGA GGG GAA CGC GAT GTT CCA ACC AAC TTG GCA TCC TTA 574
44      T   L   K   L   P   H   K   R   G   E   R   D   V   P   T   N   L   A   S   L   63
575  CCA AAA AGA GGT GAC TGC AGG TCG GGT AAT AGC AGA GGA CCT GTG AGC GGG ATC TAC CTG 634
64      P   K   R   G   D   C   R   S   G   N   S   R   W   H   N   R   L   D   C   P   L   83
635  AAG CCA GGG CCA CTA TTT TAC CAG GAC TAT AAA GGT CCC GTC TAT CAC AGG GCC CCG CTG 694
84      K   P   G   P   L   F   Y   Q   D   Y   K   G   P   V   Y   H   R   A   P   L   103
695  GAG CTC TTT GAG GAG GGA TCC ATG TGT GAA ACG ACT AAA CGG ATA GGG AGA GTA ACT GGA 754
104     E   L   F   E   E   G   S   M   C   E   T   T   K   R   I   G   R   V   T   G   123
755  AGT GAC GGA AAG CTG TAC CAC ATT TAT GTG TGT ATA GAT GGA TGT ATA ATA AAA AGT 814
124     S   D   G   K   L   Y   H   I   Y   V   C   I   D   G   C   I   I   I   K   S   143
815  GCC ACG AGA AGT TAC CAA AGG GTG TTC AGG TGG GTC CAT AAT AGG CTT GAC TGC CCT CTA 874
144     A   T   R   S   Y   Q   R   V   F   R   W   H   N   R   L   D   C   P   L   163
875  TGG GTC ACA ACT TGC TCA GAC ACG AAA GAA GAG GGA GCA ACA AAA AAG AAA ACA CAG AAA 934
164     W   V   T   T   C   S   D   T   K   E   E   G   A   T   K   K   K   T   Q   K   183
935  CCC GAC AGA CTA GAA AGG GGG AAA ATG AAA ATA GTG CCC AAA GAA TCT GAA AAA GAC AGC 994
184     P   D   R   L   E   R   G   K   M   K   I   V   P   K   E   S   E   K   D   S   203
995  AAA ACT AAA CCT CCG GAT GCT ACA ATA GTG GTG GAA GGA GTC AAA TAC CAG GTG AGG AAG 1054
204     K   T   K   P   P   D   A   T   I   V   V   E   G   V   K   Y   Q   V   R   K   223
1055  AAG GGA AAA ACC AAG AGT AAA AAC ACT CAG GAC GGC TTG TAC CAT AAC AAA AAC AAA CCT 1114
224     K   G   K   T   K   S   K   N   T   Q   D   G   L   Y   H   N   K   N   K   P   243
1115  CAG GAA TCA CGC AAG AAA CTG GAA AAA GCA TTG TTG GCG TGG GCA ATA ATA GCT ATA GTT 1174
244     Q   E   S   R   K   K   L   E   K   A   L   L   A   W   A   I   I   A   I   V   263
1175  TTG TTT CAA GTT ACA ATG GGA GAA AAC ATA ACA CAG TGG AAC CTA CAA GAT AAT GGG ACG 1234
264     L   F   Q   V   T   M   G   E   N   I   T   Q   W   N   L   Q   D   N   G   T   283
1235  GAA GGG ATA CAA CGG GCA ATG TTC CAA AGG GGT GTG AAT AGA AGT TTA CAT GGA ATC TGG 1294
284     E   G   I   Q   R   A   M   F   Q   R   G   V   N   R   S   L   H   G   I   W   303
1295  CCA GAG AAA ATC TGT ACT GGT GTC CCT TCC CAT CTA GCC ACC GAT ATA GAA CTA AAA ACA 1354
304     P   E   K   I   C   T   G   V   P   S   H   L   A   T   D   I   E   L   K   T   323
1355  ATT CAT GGT ATG ATG GAT GCA AGT GAG AAG ACC AAC TAC ACG TGT TGC AGA CTT CAA CGC 1414
324     I   H   G   M   M   D   A   S   E   K   T   N   Y   T   C   C   R   L   Q   R   343
1415  CAT GAG TGG AAC AAG CAT GGT TGG TGC AAC TGG TAC AAT ATT GAA CCC TGG ATT CTA GTC 1474
344     H   E   W   N   K   H   G   W   C   N   W   Y   N   I   E   P   W   I   L   V   363
1475  ATG AAT AGA ACC CAA GCC AAT CTC ACT GAG GGA CAA CCA CCA AGG GAG TGC GCA GTC ACT 1534
364     M   N   R   T   Q   A   N   L   T   E   G   G   P   R   E   C   A   V   T   383
1535  TGT AGG TAT GAT AGG GCT AGT GAC TTA AAC GTG GTA ACA CAA GCT AGA GAT AGC CCC ACA 1594
384     C   R   Y   D   R   A   S   D   L   N   V   V   T   Q   A   R   D   S   P   T   403
1595  CCC TTA ACA GGT TGC AAG AAA GGA AAG AAC TTC TCC TTT GCA GGC ATA TTG ATG CGG GGC 1654
404     P   L   T   G   C   K   K   G   K   N   F   S   F   A   G   I   L   M   R   G   423
1655  CCC TGC AAC TTT GAA ATA GCT GCA AGT GAT GTA TTA TTC AAA GAA CAT GAA CGC ATT AGT 1714
424     P   C   N   F   E   I   A   A   S   D   V   L   F   K   E   H   E   R   I   S   443
1715  ATG TTC CAG GAT ACT ACT CTT TAC CTT GTT GAC GGG TTG ACC AAC TCC TTA GAA GGT GCC 1774
444     M   F   Q   D   T   T   L   Y   L   V   D   G   L   T   N   S   L   E   G   A   463

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FIGURE 12-1

BVDV NADL clns- (inf. clone) Genes 31/67 4/21/99 5:45:24 PM Page 2

1775 AGA CAA GGA ACC GCT AAA CTG ACA ACC TGG TTA GGC AAG CAG CTC GGG ATA CTA GGA AAA 1836  
464 R Q G T A K L T T W L G K Q L G I L G K 483

1835 AAG TTG GAA AAC AAG AGT AAG ACG TGG TTT GGA GCA TAC GCT GCT TCC CCT TAC TGT GAT 1894  
484 K L E N K S K T W F G A Y A A S P Y C D 503

1895 GTC GAT CGC AAA ATT GGC TAC ATA TGG TAT ACA AAA AAT TGC ACC CCT GCC TGC TTA CCC 1954  
504 V D R K I G Y I W Y T K N C T P A C L P 523

1955 AAG AAC ACA AAA ATT GTC GGC CCT GGG AAA TTT GAC ACC AAT GCA GAG GAC GGC AAG ATA 2014  
524 K N T K I V G P G K F D T N A E D G K I 543

2015 TTA CAT GAG ATG GGG GGT CAC TTG TCG GAG GTA CTA CTA CTT TCT TTA GTG GTG CTG TCC 2074  
544 L H E M G G H L S E V L L L S L V V L S 563

2075 GAC TTC GCA CCG GAA ACA GCT AGT GTA ATG TAC CTA ATC CTA CAT TTT TCC ATC CCA CAA 2134  
564 D F A P E A S V W N L G K Y V C I P Q 583

2135 AGT CAC GTT GAT GTA ATG GAT TGT GAT AAG ACC CAG TTG AAC CTC ACA GTG GAG CTG ACA 2194  
584 S H V D V M D C D K T Q L N L T V E L T 603

2195 ACA GCT GAA GTA ATA CCA GGG TCG GTC TGG AAT CTA GGC AAA TAT GTA TGT ATA AGA CCA 2254  
604 T A E V I P G S V W N L G K Y V C I R P 623

2255 AAT TGG TGG CCT TAT GAG ACA ACT GTA GTG TTG GCA TTT GAA GAG GTG AGC CAG GTG GTG 2314  
624 N W W P Y E T T V L A F E E V S 643

2315 AAG TTA GTG TTG AGG GCA CTC AGA GAT TTA ACA CGC ATT TGG AAC GCT GCA ACA ACT ACT 2374  
644 K L V L R A L R D L T R I W N A A T T T 663

2375 GCT TTT TTA GTA TGC CTT GTT AAG ATA GTC AGG GGC CAG ATG GTA CAG GGC ATT CTG TGG 2434  
664 A F L V C L V K I V R G Q M V Q G I L W 683

2435 CTA CTA TTG ATA ACA GGG GTA CAA GGG CAC TTG GAT TGC AAA CCT GAA TTC TCG TAT GCC 2494  
684 L L L I T G V Q G H L D C K P E F S Y A 703

2495 ATA GCA AAG GAC GAA AGA ATT GGT CAA CTG GGG GCT GAA GGC CTT ACC ACC ACT TGG AAG 2554  
704 I A K G D E R I G L G A E G L T T W K 723

2555 GAA TAC TCA CCT GGA ATG AAG CTG GAA GAC ACA ATG GTC ATT GCT TGG TGC GAA GAT GGG 2614  
724 E Y S P G M K L E D T M V I A W C E D G 743

2615 AAG TTA ATG TAC CTC CAA AGA TGC ACG AGA GAA ACC AGG TAT CTC GCA ATC TTG CAT ACA 2674  
744 K L M Y L Q R C T R E T R Y L A I L H T 763

2675 AGA GCC TTG CCG ACC AGT GTG GTA TTC AAA AAA CTC TTT GAT GGG CGA AAG CAA GAG GAT 2734  
764 R A L P T S V V F K K L F D G R K Q E D 783

2735 GTA GTC GAA ATG AAC GAC AAT TTT GAA TTT GGA CTC TGC CCA TGT GAT GCC AAA CCC ATA 2794  
784 V V E M N D N F E F L C P C D A K P I 803

2795 GTA AGA GGG AAG TTC AAT ACA ACG CTG CTG AAC GGA CCG GCC TTC CAG ATG GTA TGC CCC 2854  
804 V R G K F N T T L L N G P A F Q M V C P 823

2855 ATA GGA TGG ACA GGG ACT GTA AGC TGT ACG TCA TTC AAT ATG GAC ACC TTA GCC ACA ACT 2914  
824 I G W T G T V S C T S F N M D T L A T T 843

2915 GTG GTA CCG ACA TAT AGA AGG TCT AAA CCA TTC CCT CAT AGG CAA GGC TGT ATC ACC CAA 2974  
844 V V R T Y R R S K P F P C G C I T Q 863

2975 AAG AAT CTG GGG GAG GAT CTC CAT AAC TGC ATC CTT GGA GGA AAT TGG ACT TGT GTG CCT 3034  
864 K N L G E D L H N C I L G G N W T C V P 883

3035 GGA GAC CAA CTA CTA TAC AAA GGG GGC TCT ATT GAA TCT TGC AAG TGG TGT GGC TAT CAA 3094  
884 G D Q L L Y K G G S I E S C K W C G Y Q 903

3095 TTT AAA GAG AGT GAG GGA CTA CCA CAC TAC CCC ATT GGC AAG TGT AAA TTG GAG AAC GAG 3154  
904 F K E S E G L P H Y P I G K C K L E N E 923

3155 ACT GGT TAC AGG CTA GTA GAC AGT ACC TCT TGC AAT AGA GAA GGT GTG GCC ATA GTA CCA 3214  
924 T G Y R L V D S C N R E G V A I V P 943

3215 CAA GGG ACA TTA AAG TGC AAG ATA GGA AAA ACA ACT GTA CAG GTC ATA GCT ATG GAT ACC 3274  
944 Q G T L K C K I G K T T V Q V I A M D T 963

3275 AAA CTC GGA CCT ATG CCT TGC AGA CCA TAT GAA ATC ATA TCA AGT GAG GGG CCT GTA GAA 3334  
964 K L G P M P C R P Y E I I S S E G P V E 983

3335 AAG ACA GCG TGT ACT TTC AAC TAC ACT AAG ACA TTA AAA AAT AAG TAT TTT GAG CCC AGA 3394  
984 K T A C T F N Y T K T L K N K Y F E P R 1003

3395 GAC AGC TAC TTT CAG CAA TAC ATG CTA AAA GGA GAG TAT CAA TAC TGG TTT GAC CTG GAG 3454  
1004 D S Y F Q Q Y M L K G E Y Q Y W F D L E 1023

3455 GTG ACT GAC CAT CAC CCG GAT TAC TTC GCT GAG TCC ATA TTA GTG GTG GTA GTA GCC CTC 3514  
1024 V T D H H R D Y F A E S I L V V V V A L 1043

FIGURE 12-2

BVDV NADL cins- (inf. clone)		Genes		32/67		4/21/99		5:45:24 PM		Page 3	
3515	TTG GGT GGC AGA TAT GTA CTT TGG TTA CTG GTT ACA TAC ATG GTC TTA TCA GAA CAG AAG	1044	L G G R Y V L W L V T Y M V L S E Q K	3574	1063						
3575	GCC TTA GGG ATT CAG TAT GGA TCA GGG GAA GTG GTG ATG ATG GGC AAC TTT CTA ACC CAT	1064	A L G I Q Y G S G E V V M M G N L T T H	3634	1083						
3635	AAC AAT ATT GAA GTG GTG ACA TAC TTC TTG CTG CTG TAC CTA CTG CTG AGG GAG GAG AGC	1084	N N I E V V T Y F L L L Y L L L R E E S	3694	1103						
3695	GTA AAG AAG TGG GTC TTA CTC TTA TAC CAC ATC TTA GTG GTA CAC CCA ATC AAA TCT GTA	1104	V K K W V L L L Y H I L V V H P I K S V	3754	1123						
3755	ATT GTG ATC CTA CTG ATG ATT GGG GAT GTG GTA AAG GCC GAT TCA GGG GGC CAA GAG TAC	1124	I V I L L M I G D V V K A D S G Q E Y	3814	1143						
3815	TTG GGG AAA ATA GAC CTC TGT TTT ACA ACA GTA GTA CTA ATC GTC ATA GGT TTA ATC ATA	1144	L G K I D L C F T T V V L I V I G L I I	3874	1163						
3875	GCC AGG CGT GAC CCA ACT ATA GTG CCA CTG GTA ACA ATA ATG GCA GCA CTG AGG GTC ACT	1164	A R R D P T I V P L V T I M A A L R V T	3934	1183						
3935	GAA CTG ACC CAC CAG CCT GGA GTT GAC ATC GCT GTG GCG GTC ATG ACT ATA ACC CTA CTG	1184	E L T H Q P G V D I A V A V M T I T L L	3994	1203						
3995	ATG GTT AGC TAT GTG ACA GAT TAT TTT AGA TAT AAA AAA TGG TTA CAG TGC ATT CTC AGC	1204	M V S Y V T D Y F R Y K K W L Q C I L S	4054	1223						
4055	CTG GTA TCT GCG GTG TTC TTG ATA AGA AGC CTA ATA TAC CTA GGT AGA ATC GAG ATG CCA	1224	L V S A V F L I R S L I Y L G R I E M P	4114	1243						
4115	GAG GTA ACT ATC CCA AAC TGG AGA CCA CTA ACT TTA ATA CTA TTA TAT TTG ATC TCA ACA	1244	E V T I P N W R P L T L I L L Y L I S T	4174	1263						
4175	ACA ATT GTA ACG AGG TGG AAG GTT GAC GTG GCT GGC CTA TTG TTG CAA TGT GTG CCT ATC	1264	T I V T R W K V D V A G L L L Q C V P I	4234	1283						
4235	TTA TTG CTG GTC ACA ACC TTG TGG GCC GAC TTC TTA ACC CTA ATA CTG ATC CTG CCT ACC	1284	L L L L V T T L W A D F L T L I L I L P T	4294	1303						
4295	TAT GAA TTG GTT AAA TTA TAC TAT CTG AAA ACT GTT AGG ACT GAT ATA GAA AGA AGT TGG	1304	Y E L V K L Y Y L K T V R T D I E R S W	4354	1323						
4355	CTA GGG GGG ATA GAC TAT ACA AGA GTT GAC TCC ATC TAC GAC GTT GAT GAG AGT GGA GAG	1324	L G G G I D Y T R V D S I Y D V D E S G E	4414	1343						
4415	GGC GTA TAT CTT TTT CCA TCA AGG CAG AAA GCA CAG GGG AAT TTT TCT ATA CTC TTG CCC	1344	G V Y L F P S R Q K A Q G G N F S I L L P	4474	1363						
4475	CTT ATC AAA GCA ACA CTG ATA AGT TGC GTC AGC AGT AAA TGG CAG CTA ATA TAC ATG AGT	1364	L I K A T L I S C V S S K W Q L I Y M S	4534	1383						
4535	TAC TTA ACT TTG GAC TTT ATG TAC TAC ATG CAC AGG AAA GTT ATA GAA GAG ATC TCA GGA	1384	Y L T L D F M Y Y M H R K V I E E I S G	4594	1403						
4595	GGT ACC AAC ATA ATA TCC AGG TTA GTG GCA GCA CTC ATA GAG CTG AAC TGG TCC ATG GAA	1404	G T N I I S R L V A A L I E L N W S M E	4654	1423						
4655	GAA GAG GAG AGC AAA GGC TTA AAG AAG TTT TAT CTA TTG TCT GGA AGG TTG AGA AAC CTA	1424	E E E S K G L K K F Y L L S G R L R N L	4714	1443						
4715	ATA ATA AAA CAT AAG GTA AGG AAT GAG ACC GTG GCT TCT TGG TAC GGG GAG GAG GAA GTC	1444	I I K H K V R N E T V A S W Y G E E E V	4774	1463						
4775	TAC GGT ATG CCA AAG ATC ATG ACT ATA ATC AAG GCC AGT ACA CTG AGT AAG AGC AGG CAC	1464	Y G M P K I M T I I K A S T L S K S R H	4834	1483						
4835	TGC ATA ATA TGC ACT GTA TGT GAG GGC CGA GAG TGG AAA GTT GGC ACC TGC CCA AAA TGT	1484	C I I C T V C E G G R E W K G G T C P K C	4894	1503						
4895	GGA CGC CAT GGG AAG CCG ATA ACG TGT GGG ATG TCG CTA GCA GAT TTT GAA GAA AGA CAC	1504	G R H G K P I T C G M S L A D F E E R H	4954	1523						
4955	TAT AAA AGA ATC TTT ATA AGG GAA GGC AAC TTT GAG gggccc TTC AGG CAG GAA TAC AAT	1524	Y K R I F I R E G N F E F R Q E Y N	5014	1541						
5015	GGC TTT GTA CAA TAT ACC GCT AGG GGG CAA CTA TTT CTG AGA AAC TTG CCC GTA CTG GCA	1542	G F V Q Y T A R G Q L F L R N L P V L A	5074	1561						
5075	ACT AAA GTA AAA ATG CTC ATG GTA GGC AAC CTT GGA GAA GAA ATT GGT AAT CTG GAA CAT	1562	T K V K M L M V G N L G E E I G N L E H	5134	1581						
5135	CTT GGG TGG ATC CTA AGG GGG CCT GCC GTG TGT AAG AAG ATC ACA GAG CAC GAA AAA TGC	1582	L G W I L R G P A V C K K I T E H E K C	5194	1601						
5195	CAC ATT AAT ATA CTG GAT AAA CTA ACC GCA TTT TTC GGG ATC ATG CCA AGG GGG ACT ACA	1602	H I N I L D K L T A F F G I M P R G T T	5254	1621						

FIGURE 12-3

FIGURE 12-3

BVDV NADL cIns- (inf. clone)		Genes		33/67		4/21/99		5:45:24 PM		Page 4											
5255	CCC	AGA	GCC	CCG	GTG	AGG	TTC	CCT	ACG	AGC	TTA	CTA	AAA	GTG	AGG	AGG	GGT	CTG	GAG	ACT	5314
1622	P	R	A	P	V	R	F	P	T	S	L	L	K	V	R	R	G	L	E	T	1641
5315	GCC	TGG	GCT	TAC	ACA	CAC	CAA	GGC	GGG	ATA	AGT	TCA	GTC	GAC	CAT	GTA	ACC	GCC	GGA	AAA	5374
1642	A	W	A	Y	T	H	Q	G	G	I	S	S	V	D	H	V	T	A	G	K	1661
5375	GAT	CTA	CTG	GTC	TGT	GAC	AGC	ATG	GGA	CGA	ACT	AGA	GTG	GTT	TGC	CAA	AGC	AAC	AAC	AGG	5434
1662	D	L	L	V	C	D	S	M	G	R	T	R	V	V	C	Q	S	N	N	R	1681
5435	TTG	ACC	GAT	GAG	ACA	GAG	TAT	GGC	GTC	AAG	ACT	GAC	TCA	GGG	TGC	CCA	GAC	GGT	GCC	AGA	5494
1682	L	T	D	E	T	E	Y	G	V	K	T	D	S	G	C	P	D	G	A	R	1701
5495	TGT	TAT	GTG	TTA	AAT	CCA	GAG	GCC	GTT	AAC	ATA	TCA	GGA	TCC	AAA	GGG	GCA	GTC	GTT	CAC	5554
1702	C	Y	V	L	N	P	E	A	V	N	I	S	G	S	K	G	A	V	V	H	1721
5555	CTC	CAA	AAG	ACA	GGT	GGA	GAA	TTC	ACG	TGT	GTC	ACC	GCA	TCA	GGC	ACA	CCG	GCT	TTC	TTC	5614
1722	L	Q	K	T	G	G	E	F	T	C	V	T	A	S	G	T	P	A	F	F	1741
5615	GAC	CTA	AAA	AAC	TTG	AAA	GGA	TGG	TCA	GGC	TTG	CCT	ATA	TTT	GAA	GCC	TCC	AGC	GGG	AGG	5674
1742	D	L	K	N	L	K	G	W	S	G	L	P	I	F	E	A	S	S	G	R	1761
5675	GTG	GTT	GGC	AGA	GTC	AAA	GTA	GGG	AAG	AAT	GAA	GAG	TCT	AAA	CCT	ACA	AAA	ATA	ATG	AGT	5734
1762	V	V	G	R	V	K	V	G	K	N	E	E	S	K	P	T	K	I	M	S	1781
5735	GGA	ATC	CAG	ACC	GTC	TCA	AAA	AAC	AGA	GCA	GAC	CTG	ACC	GAG	ATG	GTC	AAG	AAG	ATA	ACC	5794
1782	G	I	Q	T	V	S	K	N	R	A	D	L	T	E	M	V	K	K	I	T	1801
5795	AGC	ATG	AAC	AGG	GGA	GAC	TTC	AAG	CAG	ATT	ACT	TTG	GCA	ACA	GGG	GCA	GGC	AAA	ACC	ACA	5854
1802	S	M	N	R	G	D	F	K	Q	I	T	L	A	T	G	A	G	K	T	T	1821
5855	GAA	CTC	CCA	AAA	GCA	GTT	ATA	GAG	GAG	ATA	GGA	AGA	CAC	AAG	AGA	GTA	TTA	GTT	CTT	ATA	5914
1822	E	L	P	K	A	V	I	E	E	I	G	R	H	K	R	V	L	V	L	I	1841
5915	CCA	TTA	AGG	GCA	GCG	GCA	GAG	TCA	GTC	TAC	CAG	TAT	ATG	AGA	TTG	AAA	CAC	CCA	AGC	ATC	5974
1842	P	L	R	A	A	A	E	S	V	Y	Q	Y	M	R	L	K	H	P	S	I	1861
5975	TCT	TTT	AAC	CTA	AGG	ATA	GGG	GAC	ATG	AAA	GAG	GGG	GAC	ATG	GCA	ACC	GGG	ATA	ACC	TAT	6034
1862	S	F	N	L	R	I	G	D	M	K	E	G	D	M	A	T	G	I	T	Y	1881
6035	GCA	TCA	TAC	GGG	TAC	TTC	TGC	CAA	ATG	CCT	CAA	CCA	AAG	CTC	AGA	GCT	GCT	ATG	GTA	GAA	6094
1882	A	S	Y	T	G	Y	F	C	Q	M	P	Q	P	K	L	R	A	A	M	V	1901
6095	TAC	TCA	TAC	ATA	TTC	TTA	GAT	GAA	TAC	CAT	TGT	GCC	ACT	CCT	GAA	CAA	CTG	GCA	ATT	ATC	6154
1902	Y	S	Y	I	F	L	D	E	Y	H	C	A	T	P	E	Q	L	A	I	I	1921
6155	GGG	AAG	ATC	CAC	AGA	TTT	TCA	GAG	AGT	ATA	AGG	GTT	GTC	GCC	ATG	ACT	GCC	ACG	CCA	GCA	6214
1922	G	K	I	H	R	F	S	E	S	I	R	V	V	A	M	T	A	T	P	A	1941
6215	GGG	TGG	GTG	ACC	ACA	ACA	GGT	CAA	AAG	CAC	CCA	ATA	GAG	GAA	TTT	ATA	GCC	CCC	GAG	GTA	6274
1942	G	S	V	T	T	T	G	Q	K	H	P	I	E	E	F	I	A	P	E	V	1961
6275	ATG	AAA	GGG	GAG	GAT	CTT	GGT	AGT	CAG	TTC	CTT	GAT	ATA	GCA	GGG	TTA	AAA	ATA	CCA	GTG	6334
1962	M	K	G	E	D	L	G	S	Q	F	L	D	I	A	G	L	K	I	P	V	1981
6335	GAT	GAG	ATG	AAA	GGC	AAT	ATG	TTG	GTT	TTT	GTA	CCA	ACG	AGA	AAC	ATG	GCA	GTA	GAG	GTA	6394
1982	D	E	M	K	G	N	M	L	V	F	V	P	T	R	N	M	A	V	E	V	2001
6395	GCA	AAG	AAG	CTA	AAA	GCT	AAG	GGC	TAT	AAC	TCT	GGA	TAC	TAT	TAC	AGT	GGA	GAG	GAT	CCA	6454
2002	A	K	K	L	K	A	K	G	Y	N	S	G	Y	Y	Y	S	G	E	D	P	2021
6455	GCC	AAT	CTG	AGA	GTT	GTG	ACA	TCA	CAA	TCC	CCC	TAT	GTA	ATC	GTG	GCT	ACA	AAT	GCT	ATT	6514
2022	A	N	L	R	V	V	T	S	Q	S	P	Y	V	I	V	A	T	N	A	I	2041
6515	GAA	TCA	GGA	GTG	ACA	CTA	CCA	GAT	TTG	GAC	ACG	GTT	ATA	GAC	ACG	GGG	TTG	AAA	TGT	GAA	6574
2042	E	S	G	V	T	L	P	D	L	D	T	V	I	D	T	G	L	K	C	E	2061
6575	AAG	AGG	GTG	AGG	GTA	TCA	TCA	AAG	ATA	CCC	TTC	ATC	GTA	ACA	GGC	CTT	AAG	AGG	ATG	GCC	6634
2062	K	R	V	R	V	S	S	K	I	P	F	I	V	T	G	L	K	R	M	A	2081
6635	GTG	ACT	GTG	GGT	GAG	CAG	GCG	CAG	CGT	AGG	GGC	AGA	GTA	GGT	AGA	GTG	AAA	CCC	GGG	AGG	6694
2082	V	T	V	G	E	Q	A	Q	R	R	G	R	V	G	R	V	K	P	G	R	2101
6695	TAT	TAT	AGG	AGC	CAG	GAA	ACA	GCA	ACA	GGG	TCA	AAG	GAC	TAC	CAC	TAT	GAC	CTC	TTG	CAG	6754
2102	Y	Y	R	S	Q	E	T	A	T	G	S	K	D	Y	H	Y	D	L	L	Q	2121
6755	GCA	CAA	AGA	TAC	GGG	ATT	GAG	GAT	GGA	ATC	AAC	GTG	ACG	AAA	TCC	TTT	AGG	GAG	ATG	AAT	6814
2122	A	Q	R	Y	G	I	E	D	G	I	N	V	T	K	S	F	R	E	M	N	2141
6815	TAC	GAT	TGG	AGC	CTA	TAC	GAG	GAG	GAC	AGC	CTA	CTA	ATA	ACC	CAG	CTG	GAA	ATA	CTA	AAT	6874
2142	Y	D	W	S	L	Y	E	E	D	S	L	L	I	T	Q	L	E	I	L	N	2161
6875	AAT	CTA	CTC	ATC	TCA	GAA	GAC	TTG	CCA	GCC	GCT	GTT	AAG	AAC	ATA	ATG	GCC	AGG	ACT	GAT	6934
2162	N	L	L	I	S	E	D	L	P	A	A	V	K	N	I	M	A	R	T	D	2181
6935	CAC	CCA	GAG	CCA	ATC	CAA	CTT	GCA	TAC	AAC	AGC	TAT	GAA	GTC	CAG	GTC	CCG	GTC	CTG	TTC	6994
2182	H	P	E	P	I	Q	L	A	Y	N	S	Y	E	V	Q	V	P	V	L	F	2201

FIGURE 12-4

FIGURE 12-4

BVDV NADL cins- (inf. clone)

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6995 CCA AAA ATA AGG AAT GGA GAA GTC ACA GAC ACC TAC GAA AAT TAC TCG TTT CTA AAT GCC 7054
2202 P K I R N G E V T D T Y E N Y S F L N A 2221

7055 AGA AAG TTA GGG GAG GAT GTG CCC GTG TAT ATC TAC GCT ACT GAA GAT GAG GAT CTG GCA 7114
2222 R K L G E D V P V Y I Y A T E D E D L A 2241

7115 GTT GAC CTC TTA GGG CTA GAC TGG CCT GAT CCT GGG AAC CAG CAG GTA GTG GAG ACT GGT 7174
2242 V D L L G L D W P D P G N Q Q V V E T G 2261

7175 AAA GCA CTG AAG CAA GTG ACC GGG TTG TCC TCG GCT GAA AAT GCC CTA CTA GTG GCT TTA 7234
2262 K A L K Q V T G L S S A E N A L L V A L 2281

7235 TTT GGG TAT GTG GGT TAC CAG GCT CTC TCA AAG AGG CAT GTC CCA ATG ATA ACA GAC ATA 7294
2282 F G Y V G Y Q A L S K R H V P M I T D I 2301

7295 TAT ACC ATC GAG GAC CAG AGA CTA GAA GAC ACC ACC CAC CTC CAG TAT GCA CCC AAC GCC 7354
2302 Y T I E D C R L E D T T H L Q Y A P N A 2321

7355 ATA AAA ACC GAT GGG ACA GAG ACT GAA CTG AAA GAA CTG GCG TCG GGT GAC GTG GAA AAA 7414
2322 I K T D G T E T E L K E L A S G D V E K 2341

7415 ATC ATG GGA GCC ATT TCA GAT TAT GCA GCT GGG GGA CTG GAG TTT GTT AAA TCC CAA GCA 7474
2342 I M G A I S D Y A A G G L E F V K S Q A 2361

7475 GAA AAG ATA AAA ACA GCT CCT TTG TTT AAA GAA AAC GCA GAA GCC GCA AAA GGG TAT GTC 7534
2362 E K I K T A P L F K E N A E A A K G Y V 2381

7535 CAA AAA TTC ATT GAC TCA TTA ATT GAA AAT AAA GAA GAA ATA ATC AGA TAT GGT TTG TGG 7594
2382 Q K F I D S L I E N K E I I R Y G L W 2401

7595 GGA ACA CAC ACA GCA CTA TAC AAA AGC ATA GCT GCA AGA CTG GGG CAT GAA ACA GCG TTT 7654
2402 G T H T A L Y K S I A A R L G H E T A F 2421

7655 GCC ACA CTA GTG TTA AAG TGG CTA GCT TTT GGA GGG GAA TCA GTG TCA GAC CAC GTC AAG 7714
2422 A T L V L K W L A F G G E S V S D H V K 2441

7715 CAG GCG GCA GTT GAT TTA GTG GTC TAT TAT GTG ATG AAT AAG CCT TCC TTC CCA GGT GAC 7774
2442 Q A V D L V Y V M N K P S F P G D 2461

7775 TCC GAG ACA CAG CAA GAA GGG AGG CGA TTC GTC GCA AGC CTG TTC ATC TCC GCA CTG GCA 7834
2462 S E T Q Q E G R R F V A S L F I S A L A 2481

7835 ACC TAC ACA TAC AAA ACT TGG AAT TAC CAC AAT CTC TCT AAA GTG GTG GAA CCA GCC CTG 7894
2482 T Y T Y K T W N Y H N L S K V V E P A L 2501

7895 GCT TAC CTC CCC TAT GCT ACC AGC GCA TTA AAA ATG TTC ACC CCA ACG CGG CTG GAG AGC 7954
2502 A Y L P Y A T S A L K M F T P T R L E S 2521

7955 GTG GTG ATA CTG AGC ACC ACG ATA TAT AAA ACA TAC CTC TCT ATA AGG AAG GGG AAG AGT 8014
2522 V I L L Y K T I Y K T Y L S I R K K S 2541

8015 GAT GGA TTG CTG GGT ACG GGG ATA AGT GCA GCC ATG GAA ATC CTG TCA CAA AAC CCA GTA 8074
2542 D G L L G T G I S A A M E I L S Q N P V 2561

8075 TCG GTA GGT ATA TCT GTG ATG TTG GGG GTA GGG GCA ATC GCT GCG CAC AAC GCT ATT GAG 8134
2562 S V G I S V M L G V G A I A A H N A I E 2581

8135 TCC AGT GAA CAG AAA AGG ACC CTA CTT ATG AAG GTG TTT GTA AAG AAC TTC TTG GAT CAG 8194
2582 S S E Q K R T L L M K V F V K N F L D Q 2601

8195 GCT GCA ACA GAT GAG CTG GTA AAA GAA AAC CCA GAA AAA ATT ATA ATG GCC TTA TTT GAA 8254
2602 A A T D E L V K E N P E K I I M A L F E 2621

8255 GCA GTC CAG ACA ATT GGT AAC CCC CTG AGA CTA ATA TAC CAC CTG TAT GGG GTT TAC TAC 8314
2622 A V Q T I G N P L R L I Y H L Y G V Y Y 2641

8315 AAA GGT TGG GAG GCC AAG GAA CTA TCT GAG AGG ACA GCA GGC AGA AAC TTA TTC ACA TTG 8374
2642 K G W E A K E L S E R T A G R N L F T L 2661

8375 ATA ATG TTT GAA GCC TTC GAG TTA TTA GGG ATG GAC TCA CAA GGG AAA ATA AGG AAC CTG 8434
2662 I M F E A F E L L G M D S Q K I R N L 2681

8435 TCC GGA AAT TAC ATT TTG GAT TTG ATA TAC GGC CTA CAC AAG CAA ATC AAC AGA GGG CTG 8494
2682 S G N Y I L D L I Y G L H K Q I N R G L 2701

8495 AAG AAA ATG GTA CTG GGG TGG GCC CCT GCA CCC TTT AGT TGT GAC TGG ACC CCT AGT GAC 8554
2702 K K M V L G W A P A P F S C D W T P S D 2721

8555 GAG AGG ATC AGA TTG CCA ACA GAC AAC TAT TTG AGG GTA GAA ACC AGG TGC CCA TGT GGC 8614
2722 E R I R L P T D N Y L R V E T R C P C G 2741

8615 TAT GAG ATG AAA GCT TTC AAA AAT GTA GGT GGC AAA CTT ACC AAA GTG GAG GAG AGC GGG 8674
2742 Y E M K A F K N V G G K L T K V E E S G 2761

8675 CCT TTC CTA TGT AGA AAC AGA CCT GGT AGG GGA CCA GTC AAC TAC AGA GTC ACC AAG TAT 8734
2762 P F L C R N R P G R G P V N Y R V T K Y 2781

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FIGURE 12-5

BVDV NADL cIns- (inf. clone) Genes 35/67 4/21/99 5:45:24 PM Page 6

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8735 TAC GAT GAC AAC CTC AGA GAG ATA AAA CCA GTA GCA AAG TTG GAA GGA CAG GTA GAG CAC 8794
2782 Y D D N L R E I K P V A K L E G Q V E H 2801

8795 TAC TAC AAA GGG GTC ACA GCA AAA ATT GAC TAC AGT AAA GGA AAA ATG CTC TTG GCC ACT 8854
2802 Y Y K G V T A K I D Y S K G K M L L A T 2821

8855 GAC AAG TGG GAG GTG GAA CAT GGT GTC ATA ACC AGG TTA GCT AAG AGA TAT ACT GGG GTC 8914
2822 D K W E V E H G V I T R L A K R Y T G V 2841

8915 GGG TTC AAT GGT GCA TAC TTA GGT GAC GAG CCC AAT CAC CGT GCT CTA GTG GAG AGG GAC 8974
2842 G F N G A Y L G D E P N H R A L V E R D 2861

8975 TGT GCA ACT ATA ACC AAA AAC ACA GTA CAG TTT CTA AAA ATG AAG AAG GGG TGT GCG TTC 9034
2862 C A T I T K N T V Q F L K M K K G C A F 2881

9035 ACC TAT GAC CTG ACC ATC TCC AAT CTG ACC AGG CTC ATC GAA CTA GTA CAC AGG AAC AAT 9094
2882 T Y D L T I S N L T R L I E L V H R N N 2901

9095 CTT GAA GAG AAG GAA ATA CCC ACC GCT ACG GTC ACC ACA TGG CTA GCT TAC ACC TTC GTG 9154
2902 L E E K E I P T A T V T T W L A Y T F V 2921

9155 AAT GAA GAC GTA GGG ACT ATA AAA CCA GTA CTA GGA GAG AGA GTA ATC CCC GAC CCT GTA 9214
2922 N E D V G T I K P V L G E R V I P D P V 2941

9215 GTT GAT ATC AAT TTA CAA CCA GAG GTG CAA GTG GAC ACG TCA GAG GTT GGG ATC ACA ATA 9274
2942 V D I N L T K P E V Q V D T S E V G I T I 2961

9275 ATT GGA AGG GAA ACC CTG ATG ACA ACG GGA GTG ACA CCT GTC TTG GAA AAA GTA GAG CCT 9334
2962 I G R E T L M T T G V T P V L E K V E P 2981

9335 GAC GCC AGC GAC AAC CAA AAC TCG GTG AAG ATC GGG TTG GAT GAG GGT AAT TAC CCA GGG 9394
2982 D A S D N Q N S V K I G L D E G N Y P G 3001

9395 CCT GGA ATA CAG ACA CAT ACA CTA ACA GAA GAA ATA CAC AAC AGG GAT GCG AGG CCC TTC 9454
3002 P G I Q T H T L T E E I H N R D A R P F 3021

9455 ATC ATG ATC CTG GGC TCA AGG AAT TCC ATA TCA AAT AGG GCA AAG ACT GCT AGA AAT ATA 9514
3022 I M I L G S R N S I S N R A K T A R N I 3041

9515 AAT CTG TAC ACA GGA AAT GAC CCC AGG GAA ATA CGA GAC TTG ATG GCT GCA GGG CGC ATG 9574
3042 N L Y T G N D P R E I R D L M A A G R M 3061

9575 TTA GTA GTA GCA CTG AGG GAT GTC GAC CCT GAG CTG TCT GAA ATG GTC GAT TTC AAG GGG 9634
3062 L V V A L R D V D P E L S E M V D F K G 3081

9635 ACT TTT TTA GAT AGG GAG GCC CTG GAG GCT CTA AGT CTC GGG CAA CCT AAA CCG AAG CAG 9694
3082 T F L D R E A L G S I S L G Q P K Q 3101

9695 GTT ACC AAG GAA GCT GTT AGG AAT TTG ATA GAA CAG AAA AAA GAT GTG GAG ATC CCT AAC 9754
3102 V T K E A V R N L I E Q K K D V E I P N 3121

9755 TGG TTT GCA TCA GAT GAC CCA GTA TTT CTG GAA GTG GCC TTA AAA AAT GAT AAG TAC TAC 9814
3122 W F A S D D P V F L E V A L K N D K Y Y 3141

9815 TTA GTA GGA GAT GTT GGA GAG CTA AAA GAT CAA GCT AAA GCA CTT GGG GCC ACG GAT CAG 9874
3142 L V G D V G E L K D Q A K A L G A T D Q 3161

9875 ACA AGA ATT ATA AAG GAG GTA GGC TCA AGG ACG TAT GCC ATG AAG CTA TCT AGC TGG TTC 9934
3162 T R I I K E V G S R T Y A M K L S S W F 3181

9935 CTC AAG GCA TCA AAC AAA CAG ATG AGT TTA ACT CCA CTG TTT GAG GAA TTG TTG CTA CGG 9994
3182 L K A S N K Q M S L T P L F E E L L L R 3201

9995 TGC CCA CCT GCA ACT AAG AGC AAT AAG GGG CAC ATG GCA TCA GCT TAC CAA TTG GCA CAG 10054
3202 C P P A T K S N K G H M A S A Y Q L A Q 3221

10055 GGT AAC TGG GAG CCC CTC GGT TGC GGG GTG CAC CTA GGT ACA ATA CCA GCC AGA AGG GTG 10114
3222 G N W E P L G C G V H L G T T I P A R V 3241

10115 AAG ATA CAC CCA TAT GAA GCT TAC CTG AAG TTG AAA GAT TTC ATA GAA GAA GAG AAG 10174
3242 K I H P Y E A Y L K L K D F I E E E K 3261

10175 AAA CCT AGG GTT AAG GAT ACA GTA ATA AGA GAG CAC AAC AAA TGG ATA CTT AAA AAA ATA 10234
3262 K P R V K D T V I R E H N K W I L K K I 3281

10235 AGG TTT CAA GGA AAC CTC AAC ACC AAG AAA ATG CTC AAC CCG GGG AAA CTA TCT GAA CAG 10294
3282 R F Q G N L N T K K M L N P G K L S E Q 3301

10295 TTG GAC AGG GAG GGG CGC AAG AGG AAC ATC TAC AAC CAC CAG ATT GGT ACT ATA ATG TCA 10354
3302 L D R E G R K R I Y N H Q I G T I M S 3321

10355 AGT GCA GGC ATA AGG CTG GAG AAA TTG CCA ATA GTG AGG GCC CAA ACC GAC ACC AAA ACC 10414
3322 S A G I R L E K L P I V R A Q T D T K T 3341

10415 TTT CAT GAG GCA ATA AGA GAT AAG ATA GAC AAG AGT GAA AAC CGG CAA AAT CCA GAA TTG 10474
3342 F H E A I R D K I D K S E N R Q N P E L 3361

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FIGURE 12-6

BVDV NADL cins- (inf. clone)		Genes		36/67		4/21/99		5:45:24 PM		Page 7	
10475	CAC AAC AAA	TTG TTG GAG ATT TTC CAC ACG ATA GCC CAA CCC ACC CTG AAA CAC ACC TAC	10534								
3362	H N K L L E I F H T I A Q P T L K H T Y		3381								
10535	GGT GAG GTG ACG TGG GAG CAA CTT GAG GCG GGG ATA AAT AGA AAG GGG GCA GCA GGC TTC	10594									
3382	G E V T W E Q L E A G I N R K G A A G F		3401								
10595	CTG GAG AAG AAG AAC ATC GGA GAA GTA TTG GAT TCA GAA AAG CAC CTG GTA GAA CAA TTG	10654									
3402	L E K K N I G E V L D S E K H L V E Q L		3421								
10655	GTC AGG GAT CTG AAG GCC GGG AGA AAG ATA AAA TAT TAT GAA ACT GCA ATA CCA AAA AAT	10714									
3422	V R D L K A G R K I K Y Y E T A I P K N		3441								
10715	GAG AAG AGA GAT GTC AGT GAT GAC TGG CAG GCA GGG GAC CTG GTG GTT GAG AAG AGG CCA	10774									
3442	E K R D V S D D W Q A G D L V V E K R P		3461								
10775	AGA GTT ATC CAA TAC CCT GAA GCC AAG ACA AGG CTA GCC ATC ACT AAG GTC ATG TAT AAC	10834									
3462	R V I Q Y P E A K T R L A I T K V M Y N		3481								
10835	TGG GTG AAA CAG CAG CCC GTT GTG ATT CCA GGA TAT GAA GGA AAG ACC CCC TTG TTC AAC	10894									
3482	W V K Q Q P V V I P G Y E G K T P L F N		3501								
10895	ATC TTT GAT AAA GTG AGA AAG GAA TGG GAC TCG TTC AAT GAG CCA GTG GCC GTA AGT TTT	10954									
3502	I F D K V R K E W D S F N E P V A V S F		3521								
10955	GAC ACC AAA GCC TGG GAC ACT CAA GTG ACT AGT AAG GAT CTG CAA CTT ATT GGA GAA ATC	11014									
3522	D T K A A V D T Q V T S K D L Q L I G E I		3541								
11015	CAG AAA TAT TAC TAT AAG AAG GAG TGG CAC AAG TTC ATT GAC ACC ATC ACC GAC CAC ATG	11074									
3542	Q K Y Y Y K K E W H K F I D T I T D H M		3561								
11075	ACA GAA GTA CCA GTT ATA ACA GCA GAT GGT GAA GTA TAT ATA AGA AAT GGG CAG AGA GGG	11134									
3562	T E V P V I T A D G E V Y I R N G Q R G		3581								
11135	AGC GGC CAG CCA GAC ACA AGT GCT GGC AAC AGC ATG TTA AAT GTC CTG ACA ATG ATG TAC	11194									
3582	S G Q P D T S A G N S M L N V L T M M Y		3601								
11195	GGC TTC TGC GAA AGC ACA GGG GTA CCG TAC AAG AGT TTC AAC AGG GTG GCA AGG ATC CAC	11254									
3602	G F C E S T G V P Y K S F N R V A R I H		3621								
11255	GTC TGT GGG GAT GAT GGC TTC TTA ATA ACT GAA AAA GGG TTA GGG CTG AAA TTT GCT AAC	11314									
3622	V C G D D G F L I T E K G L G L K F A N		3641								
11315	AAA GGG ATG CAG ATT CTT CAT GAA GCA GGC AAA CCT CAG AAG ATA ACG GAA GGG GAA AAG	11374									
3642	K G M Q I L H E A G K P Q K I T E G E K		3661								
11375	ATG AAA GTT GCC TAT AGA TTT GAG GAT ATA GAG TTC TGT TCT CAT ACC CCA GTC CCT GTT	11434									
3662	M K V A Y R F E D I E F C S H T P V P V		3681								
11435	AGG TGG TCC GAC AAC ACC AGT AGT CAC ATG GCC GGG AGA GAC ACC GCT GTG ATA CTA TCA	11494									
3682	R W S D N T S S H M A G R D T A V I L S		3701								
11495	AAG ATG GCA ACA AGA TTG GAT TCA AGT GGA GAG AGG GGT ACC ACA GCA TAT GAA AAA GCG	11554									
3702	K M A T R L D S S G E R G T T A Y E K A		3721								
11555	GTA GCC TTC AGT TTC TTG CTG ATG TAT TCC TGG AAC CCG CTT GTT AGG AGG ATT TGC CTG	11614									
3722	V A F S F L L M Y S W N P L V R R I C L		3741								
11615	TTG GTC CTT TCG CAA CAG CCA GAG ACA GAC CCA TCA AAA CAT GCC ACT TAT TAT TAC AAA	11674									
3742	L V L S Q Q P E T D P S K H A T Y Y Y K		3761								
11675	GGT GAT CCA ATA GGG GCC TAT AAA GAT GTA ATA GGT CGG AAT CTA AGT GAA CTG AAG AGA	11734									
3762	G D P I G A Y K D V I G R N L S E L K R		3781								
11735	ACA GGC TTT GAG AAA TTG GCA AAT CTA AAC CTA AGC CTG TCC ACG TTG GGG ATC TGG ACT	11794									
3782	T G F E K L A N L N L S L S T L G I W T		3801								
11795	AAG CAC ACA AGC AAA AGA ATA ATT CAG GAC TGT GTT GCC ATT GGG AAA GAA GAG GGC AAC	11854									
3802	K H T S K R I I Q D C V A I G K E E G N		3821								
11855	TGG CTA GTT AAC GCC GAC AGG CTG ATA TCC AGC AAA ACT GGC CAC TTA TAC ATA CCT GAT	11914									
3822	W L V N A D R L I S S K T G H L Y I P D		3841								
11915	AAA GGC TTT ACA TTA CAA GGA AAG CAT TAT GAG CAA CTG CAG CTA AGA ACA GAG ACA AAC	11974									
3842	K G F T L Q G K H Y E Q L Q L R T E T N		3861								
11975	CCG GTC ATG GGG GTT GGG ACT GAG AGA TAC AAG TTA GGT CCC ATA GTC AAT CTG CTG CTG	12034									
3862	P V M G V G T E R Y K L G P I V N L L L		3881								
12035	AGA AGG TTG AAA ATT CTG CTC ATG ACG GCC GTC GGC GTC AGC AGC TGA gacaaaatgtatatat	12098									
3882	R R L K I L L M T A V G V S S		3897								
12099	tgtaataaaatcaatccatgtacatagtgatatataaatatagttgggaccgtccacctcaagaagacgacacgccccaa	12178									
12179	cgcacagctaaacagtagtcaagattatctacctcaagataaacactacatttaatgcacacagcacttttagctgtatgag	12258									
12259	gatacgccccgacgtctatagttggactagggaagacaccttaacagcccc	12308									

FIGURE 12-7

FIGURE 12-7



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GTATaatcactcccctgtgaggaactactgtcttcacgcagaaagcgtctagccatggcgtagtatgagtgtcgtgcagcctccag  
gacccccctcccgggagagccatagtggctgcggaaccggtagtacaccggaattgccaggacgaccgggtccttcttggata  
aaccgctcaatgcctggagattggcggtgccccgcaagactgtagccgagtagtgttgggtcgcgaaaggccttgggtactgc  
ctgatagggtgcttgcgagtgtcccgagggtctcgtagaccgtgcaccATG

**FIGURE 13**

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GTaatcactcccctgtgaggaactactgtcttcacgcagaaagcgtctagccatggcgtagtatgagtgtcgtgcagccctcaggac  
ccccctcccgggagagccatagtgggtctgcggaaccggtgagtacaccggaattgccaggacgaccgggtcctttcttgataaac  
ccgtcaatgcctggagatttggcgtgccccgcaagactgctagccgagtagtgttgggtcgcgaaaggccttgggtactgcctg  
atagggtgcttgcgagtccccgggaggtctcgtagaccgtgcaccATG

**FIGURE 14**

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GTATacactccacatgaatcactcccctgtgaggaactactgtcttcacgcagaaagcgtctagccatggcgttagtatgagtgcg  
tgcagcctccaggacccccctccgggagagccatagtggctcgcggaaccggtgagtacaccggaaltgccaggacgaccggg  
tcctttcttgataaacccgctcaatgcctggagattgggcgtgccccgcaagactgctagccgagtagtgttgggtcgcgaaaggc  
cttgtgtactgcctgatagggtgcttgcgagtgtccccgggaggtctcgtagaccgtgcaccATG

FIGURE 15

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GTATCAGAAGTGCGAATGCTGAacactccaccatgaatcactcccctgtgaggaactactgtcttcacgcagaaa  
gcgtctagccatggcgtagtatgagtgctgcagcctccaggacccccctcccgggagagccatagtggctgcggaaccggtg  
agtacaccggaattgccaggacgaccgggtccttcttgataaaccgctcaatgcctggagattgggcgtgccccgcaagactg  
ctagccgagtagtggtggcgcgaaaggccttggtactgcctgatagggtgcttcgagtgccccgggaggtctcgtagaccgtg  
caccATG

FIGURE 16

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GTATgccagccccctgatgggggcgacacccaccatgaatcactcccctgtgaggaactactgtcttcacgcagaaagcgtctag  
ccatggcgtagtatgagtgtcgtgcagcctccaggacccccctcccgggagagccatagtggctgcggaaccgggtgagtacacc  
ggaattgccaggacgaccgggtccttcttggataaacccgtcaatgcctggagattgggcgtgccccgcaagactgctagccga  
gtagtgtgggtcgcgaaaggccitgtggtactgcctgatagggtgcttgcgagtgtccccgggaggtctcgtagaccgtgcaccAT  
G

FIGURE 17

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GTATTGCAGTTTgccagcccctgatgggggcgacactccaccatgaatcactcccctgtgaggaactactgtcttcacgc  
agaaagcgtctagccatggcgttagtatgagtgtcgtgcagccicaggacccccctcccgggagagccatagtggctcgcggaac  
cggtagtacaccggaattgccaggacgaccgggtcctttcttgataaaccgcicaatgcctggagatttgggcgtgccccgcaa  
gactgtagccgagtagtgttgggtcgcgaaaggccttgtgtactgcctgatagggtgcttgcgagtccccgggaggtctcgtaga  
ccgtgcaccATG

**FIGURE 18**

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GTATTGCAGTTTgccagccccctgatgggggacactccaccatgaatcactccccctgtgaggaactactgtcttcacgc  
agaagcgctagccatggcgtagtagtgctgcagcctccaggacccccctccgggagagccatagtggtctgcggaac  
cggtagtacaccggaattgccaggacgaccgggtccttcttgataaaccgctcaatgcctggagattgggcgtgccccgcaa  
gactgtagccgagtagtgggtcgcgaaaggccttggtgactgcctgatagggtgcttgcgagtgccccgggaggtctcgtaga  
ccgtgcaccATGGAGTTGATCACAATGAACTTTTATACAAAACATACAAAACAAAAAC  
CCGTCGGGGTGGAGGAACCTGTTTATGATCAGGCAGGTGATCCCTTATTTGGT  
GAAAGGGGAGCAGTCCACCCTCAATCGACGCTAAAGCTCCACACAAGAGAG  
GGGAACGCGATGTTCCAACCAACTTGGCATCCTTACCAAAAAGAGGTGACTGC  
AGGTCGGGTAATAGCAGAGGACCTGTGAGCGGGATCTACCTGAAGCCAGGGC  
CACTATTTTACCAGGACTATAAAGTCCCGTCTATCACAGGGCCCCGCTGGAGC  
TCTTTGAGGAGGGATCCATGTGTGAAACGACTAAACGGATAGGGAGAGTAACT  
GGAAGTGACGGAAGCTGTACCACATTTATGTGTGTATAGATGGATGTATAATA  
ATAAAAAGTGCCACGAGAAGTTACCAAGGGTGTTCAGGTGGGTCCATAATAG  
GCTTGACTGCCCTCTATGGGTCACTTGTCTCAGACACGAAAGAAGAGGGAG  
CAACAAAAAAGAAAAACACAGAAACCCGACAGACTAGAAAGGGGGAAAAATGAA  
AATAGTGCCCAAAGAATCTGAAAAAGACAGCAAACTAAACCTCCGGATGCTA  
CAATAGTGGTGGAAGGAGTCAAAATACCAGGTGAGGAAGAAGGGAAAAACCAA  
GAGTAAAAACACTCAGGACGGCTTGTACCATAAACAAAAACAACTCAGGAAT  
CACGCAAGAACTGGAAGAAAGCATTGTTGGCGTGGGCAATAATAGCTATAGTT  
TTGTTTCAAGTTACAATGGGAGAAAACATAACACAGTGGAACCTACAAGATAAT  
GGGACGGAAGGGATACAACGGGCAATGTTCCAAAGGGGTGTGAATAGAAGTT  
TACATGGAATCTGGCCAGAGAAAACTGTACTGGTGTCCCTTCCCCTAGCCA  
CCGATATAGAACTAAAAACAATTCATGGTATGATGGATGCAAGTGAAGAAGC  
AACTACACGTGTTGCAGACTTCAACGCCATGAGTGGAACAAGCATGGTTGGTG  
CAACTGGTACAATATTGAACCTGGATTCTAGTCATGAATAGAACCCAAGCCAA  
TCTCACTGAGGGACAACCACCAAGGGAGTGCGCAGTCACTTGTAGGTATGATA  
GGGCTAGTGACTTAAACGTGGTAACACAAGCTAGAGATAGCCCCACACCTTA  
ACAGGTTGCAAGAAAGGAAGAACTTCTCCTTTGCAGGCATATTGATGCGGGG  
CCCCTGCAACTTTGAAATAGCTGCAAGTGATGTATTATTCAAAGAACATGAACG  
CATTAGTATGTTCCAGGATACTACTCTTTTACCTTGTGACGGGTGACCAACTCC  
TTAGAAGGTGCCAGACAAGGAACCGCTAAACTGACAACCTGGTTAGGCAAGCA  
GCTCGGGATACTAGGAAAAAAGTTGGAAAAACAAGAGTAAGACGTGGTTTGGAG  
CATACGCTGCTTCCCCTTACTGTGATGTCGATCGCAAAATTGGCTACATATGGT  
ATACAAAAAATTGCACCCCTGCCTGCTTACCCAAGAACAAAAAATTGTCGGCC  
CTGGGAAATTGACACCAATGCAGAGGACGGCAAGATATTACATGAGATGGGG  
GGTCACTTGTGCGAGGTACTACTTCTTTTGTAGTGGTGCTGTCCGACTTCGCA  
CCGGAACAGCTAGTGTAATGTACCTAATCCTACATTTTTTCCATCCCAAAAGTC  
ACGTTGATGTAATGGATTGTGATAAGACCCAGTTGAACCTCACAGTGGAGCTG  
ACAACAGCTGAAGTAATACCAGGGTCTGGTCTGGAATCTAGGCAAAATATGTATG  
TATAAGACCAAATTGGTGGCCTTATGAGACAACTGTAGTGTGGCATTGGAAGA  
GGTGAGCCAGGTGGTGAAGTTAGTGTGAGGGCACTCAGAGATTAAACACGCA  
TTTGGAACGCTGCAACAACACTACTGTTTTTTAGTATGCCTTGTAAAGATAGTCAG  
GGCCAGATGGTACAGGGCATTCTGTGGCTACTATTGATAACAGGGGTACAAG  
GGCACTTGGATTGCAAACTGAATTCTCGTATGCCATAGCAAAGGACGAAAGA  
ATTGGTCAACTGGGGGCTGAAGGCCTTACCACCACTTGGGAAGGAATACTCACC  
TGGAATGAAGCTGGAAGACACAATGGTCATTGCTTGGTGCGAAGATGGGAAGT  
TAATGTACCTCCAAAGATGCACGAGAGAAACCAGGTATCTCGCAATCTTGCATA  
CAAGAGCCTTGCCGACCAGTGTGGTATTCAAAAAACTCTTTGATGGGCGAAAG

FIGURE 19-1

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CAAGAGGATGTAGTCGAAATGAACGACAACCTTTGAATTTGGACTCTGCCCATGT  
GATGCCAAACCCATAGTAAGAGGGAAGTTCAATACAACGCTGCTGAACGGACC  
GGCCTTCCAGATGGTATGCCCCATAGGATGGACAGGGACTGTAAGCTGTACGT  
CATTCAATATGGACACCTTAGCCACAACCTGTGGTACGGACATATAGAAGGTCTA  
AACCATTCCCTCATAGGCAAGGCTGTATCACCCAAAAGAATCTGGGGGAGGAT  
CTCCATAACTGCATCCTTGGAGGAAATTGGACTTGTGTGCCTGGAGACCAACTA  
CTATACAAAGGGGGCTCTATTGAATCTTGCAAGTGGTGTGGCTATCAATTTAAA  
GAGAGTGAGGGACTACCACACTACCCCATTTGGCAAGTGTAATTGGAGAACGA  
GACTGGTTACAGGCTAGTAGACAGTACCTCTTGCAATAGAGAAGGTGTGGCCA  
TAGTACCACAAGGGACATTAAAGTGCAAGATAGGAAAAACAACGTACAGGTC  
ATAGCTATGGATACCAAACCTCGGACCTATGCCTTGCAAGCATATGAAATCATA  
TCAAGTGAGGGGGCCTGTAGAAAAGACAGCGTGTACTTTCAACTACACTAAGAC  
ATTAAAAATAAGTATTTTGAGCCCAGAGACAGCTACTTTCAGCAATACATGCT  
AAAAGGAGAGTATCAATACTGGTTTGACCTGGAGGTGACTGACCATCACCGGG  
ATTACTTCGCTGAGTCCATATTAGTGGTGTAGTAGCCCTCTTGGGTGGCAGAT  
ATGTACTTTGGTTACTGGTTACATACATGGTCTTATCAGAACAAGGCCTTAG  
GGATTCAGTATGGATCAGGGGAAGTGGTGTATGATGGGCAACTTGCTAACCCAT  
AACAATATTGAAGTGGTGACATACTTCTTGCTGCTGTACCTACTGCTGAGGGAG  
GAGAGCGTAAAGAAGTGGGTCTTACTCTTATACCACATCTTAGTGGTACACCCA  
ATCAAATCTGTAATTGTGATCCTACTGATGATTGGGGATGTGGTAAAGGCCGAT  
TCAGGGGGCCAAGAGTACTTGGGGAAAAATAGACCTCTGTTTTACAACAGTAGT  
ACTAATCGTCATAGGTTAATCATAGCCAGGCGTGACCCAACCTATAGTGCCACT  
GGTAACAATAATGGCAGCACTGAGGGTCACTGAACTGACCCACCAGCCTGGAG  
TTGACATCGCTGTGGCGGTCTGACTATAACCCCTACTGATGGTTAGCTATGTGA  
CAGATTATTTTAGATATAAAAAATGGTTACAGTGCATTCTCAGCCTGGTATCTGC  
GGTGTTCTTGATAAGAAGCCTAATATACCTAGGTAGAATCGAGATGCCAGAGG  
TAACTATCCCAAACCTGGAGACCACTAACTTTAATACTATTATTTGATCTCAAC  
AACAATTGTAACGAGGTGGAAGGTTGACGTGGCTGGCCTATTGTTGCAATGTG  
TGCCTATCTTATTGCTGGTCACAACCTTGTGGGCGGACTTCTTAACCCTAATACT  
GATCCTGCCTACCTATGAATTGGTTAAATTATACTATCTGAAAACCTGTTAGGACT  
GATATAGAAAGAAGTTGGCTAGGGGGGATAGACTATACAAGAGTTGACTCCAT  
CTACGACGTTGATGAGAGTGGAGAGGGCGTATATCTTTTCCATCAAGGCAGA  
AAGCACAGGGGAATTTTCTATACTCTTGCCCTTATCAAAGCAACACTGATAA  
GTTGCGTCAGCAGTAAATGGCAGCTAATATACATGAGTTACTTAACTTTGGACT  
TTATGTACTACATGCACAGGAAAGTTATAGAAGAGATCTCAGGAGGTACCAACA  
TAATATCCAGGTTAGTGGCAGCACTATAGAGCTGAACTGGTCCATGGAAGAA  
GAGGAGAGCAAAGGCTTAAAGAAGTTTTATCTATTGTCTGGAAGGTTGAGAAA  
CCTAATAATAAAACATAAGGTAAGGAATGAGACCGTGGCTTCTTGGTACGGGG  
AGGAGGAAGTCTACGGTATGCCAAAGATCATGACTATAATCAAGGCCAGTACA  
CTGAGTAAGAGCAGGCACTGCATAATATGCACTGTATGTGAGGGCCGAGAGTG  
GAAAGGTGGCACCTGCCCAAATGTGGACGCCATGGGAAGCCGATAACGTTGT  
GGGATGTGCTAGCAGATTTTGAAGAAAGACACTATAAAAGAATCTTTATAAGG  
GAAGGCAACTTTGAGGGTATGTGCAGCCGATGCCAGGGAAAGCATAGGAGGT  
TTGAAATGGACCGGGAACCTAAGAGTGCCAGATACTGTGCTGAGTGTAAATAGG  
CTGCATCCTGCTGAGGAAGGTGACTTTTGGGCAGAGTCGAGCATGTTGGGCCT  
CAAAATCACCTACTTTGCGCTGATGGATGGAAAGGTGTATGATATCACAGAGTG  
GGCTGGATGCCAGCGTGTGGGAATCTCCCAAGATACCCACAGAGTCCCTTTGTC  
ACATCTCATTTGGTTACGGATGTCCTTTGAGGAGGAATAACAATGGCTTTGTAC  
AATATACCGCTAGGGGGCAACTATTTCTGAGAACTTGCCCGTACTGGCAACTA  
AAGTAAAAATGCTCATGGTAGGCAACCTTGGAGAAGAAATTGGTAATCTGGAA  
CATCTTGGGTGGATCCTAAGGGGGCCTGCCGTGTGTAAGAAGATCACAGAGCA  
CGAAAAATGCCACATTAATACTGGATAAACTAACCCGATTTTTCGGGATCAT  
GCCAAGGGGGACTACCCCAAGAGCCCCGGTGAGGTTCCCTACGAGCTTACTAA  
AAGTGAGGAGGGGTCTGGAGACTGCCTGGGCTTACACACACCAAGGCGGGAT

FIGURE 19-2



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AAGTTCAGTCGACCATGTAACCGCCGGAAAAAGATCTACTGGTCTGTGACAGCA  
TGGGACGAACTAGAGTGGTTTGCCAAAGCAACAACAGGTTGACCGATGAGACA  
GAGTATGGCGTCAAGACTGACTCAGGGTGCCAGACGGTGCCAGATGTTATGT  
GTTAAATCCAGAGGCCGTTAACATATCAGGATCCAAAGGGGCGAGTCGTTACCC  
TCCAAAAGACAGGTGGAGAATTCACGTGTGTACCGCATCAGGCACACCGGCT  
TTCTTCGACCTAAAAAACTTGAAAGGATGGTCAGGCTTGCCCTATATTTGAAGCC  
TCCAGCGGGAGGGTGGTTGGCAGAGTCAAAGTAGGGAAGAATGAAGAGTCTA  
AACCTACAAAAATAATGAGTGGAAATCCAGACCGTCTCAAAAAACAGAGCAGAC  
CTGACCGAGATGGTCAAGAAGATAACAGCATGAACAGGGGAGACTTCAAGCA  
GATTACTTTGGCAACAGGGGCGAGGCAAAACACAGAACTCCCAAAAGCAGTTA  
TAGAGGAGATAGGAAGACACAAGAGAGTATTAGTTCTTATACCATTAAGGGCA  
GCGGCAGAGTCAGTCTACCAGTATATGAGATTGAAACACCCAAGCATCTCTTTT  
AACCTAAGGATAGGGGACATGAAAGAGGGGGACATGGCAACCGGGGATAACCT  
ATGCATCATACGGGTACTTCTGCCAAATGCCTCAACCAAAGCTCAGAGCTGCTA  
TGGTAGAATACTCATACATATTCTTAGATGAATACCATTGTGCCACTCCTGAACA  
ACTGGCAATTATCGGGAAGATCCACAGATTTTCAGAGAGTATAAGGGTTGTCTG  
CCATGACTGCCACGCCAGCAGGGTCTGGTGACCACAACAGGTCAAAAGCACCCCA  
ATAGAGGAATTCATAGCCCCCGAGGTAATGAAAGGGGAGGATCTTGGTAGTCA  
GTTCTTTGATATAGCAGGGTTAAAAATACCAGTGGATGAGATGAAAGGCAATAT  
GTTGGTTTTTGTACCAACGAGAAACATGGCAGTAGAGGTTAGCAAAGAAGCTAA  
AAGCTAAGGGCTATAACTCTGGATACTATTACAGTGGAGAGGATCCAGCCAAT  
CTGAGAGTTGTGACATCACAATCCCCCTATGTAATCGTGGCTACAAATGCTATT  
GAATCAGAGTGACACTACCAGATTTGGACACGGTTATAGACACGGGGTTGAA  
ATGTGAAAAGAGGGTGAGGGTATCATCAAAGATACCTTTCATCGTAACAGGCC  
TTAAGAGGATGGCCGTGACTGTGGGTGAGCAGGCGCAGCGTAGGGGCGAGAGT  
AGGTAGAGTGAAACCCGGGAGGTATTATAGGAGCCAGGAAACAGCAACAGGG  
TCAAAGGACTACCACTATGACCTCTTGCCAGGCACAAAGATACGGGATTGAGGA  
TGGAATCAACGTGACGAAATCCTTTAGGGAGATGAATTACGATTGGAGCCTATA  
CGAGGAGGACAGCCTACTAATAACCCAGCTGGAAATACTAAATCTACTCAT  
CTCAGAAGACTTGCCAGCCGCTGTTAAGAACATAATGGCCAGGACTGATCACC  
CAGAGCCAATCCAACCTTGCCATACAACAGCTATGAAGTCCAGGTCCCGGTCCTGT  
TCCCAAAAATAAGGAATGGAGAAGTCACAGACACCTACGAAAATTACTCGTTTC  
TAAATGCCAGAAAGTTAGGGGAGGATGTGCCCGTGTATATCTACGCTACTGAA  
GATGAGGATCTGGCAGTTGACCTCTTAGGGCTAGACTGGCCTGATCCTGGGAA  
CCAGCAGGTAGTGAGACTGGTAAGCAAGTGAAGCAAGTGACCGGGTTGTCTCT  
CGGCTGAAAATGCCCTACTAGTGGCTTTATTTGGGTATGTGGGTACCAGGCTC  
TCTCAAAGAGGCATGTCCCAATGATAACAGACATATATACCATCGAGGACCAGA  
GACTAGAAGACACCACCCACCTCCAGTATGCACCCAACGCCATAAAAACCGAT  
GGGACAGAGACTGAACTGAAAGAACTGGCGTCCGGGTGACGTGGAAAAAATCA  
TGGGAGCCATTTAGATTATGCAGCTGGGGGACTGGAGTTTGTAAATCCCAA  
GCAGAAAAGATAAAAAACAGTCTCTTTGTTTAAAGAAAACGCAGAAAGCCGCAA  
AGGGTATGTCCAAAAATTCATTGACTCATTAATTGAAAATAAAGAAAGAAATAAT  
CAGATATGGTTTGTGGGGAACACACACAGCACTATACAAAAGCATAGCTGCAA  
GACTGGGGCATGAAACAGCGTTTGCCACACTAGTGTAAAGTGGCTAGCTTTT  
GGAGGGGAATCAGTGTGAGACCGTCAAGCAGGCGGCAGTTGATTTAGTGG  
TCTATTATGTGATGAATAAGCCTTCTTCCAGGTGACTCCGAGACACAGCAAG  
AAGGGAGGCGATTTCGTGCAAGCCTGTTCTATCTCCGCACTGGCAACCTACACA  
TACAAAAGCTTGAATTACCAATCTCTCTAAAGTGGTGGAAACCGCCTGGCT  
TACCTCCCCTATGCTACCAGCGCATTAATAAATGTTTACCCCCAACGCGGCTGGAG  
AGCGTGGTGATACTGAGCACCACGATATATAAAACATACCTCTCTATAAGGAAG  
GGGAAGAGTGATGGATTGCTGGGTACGGGGATAAGTGCAGCCATGGAAATCC  
TGTCACAAAACCCAGTATCGGTAGGTATATCTGTGATGTTGGGGGTAGGGGCA  
ATCGCTGCGCACAAACGCTATTGAGTCCAGTGAACAGAAAAGGACCCTACTTAT  
GAAGGTGTTTGTAAAGAACTTCTTGATCAGGCTGCAACAGATGAGCTGGTAA

FIGURE 19-3

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AAGAAAACCCAGAAAAAATTATAATGGCCTTATTTGAAGCAGTCCAGACAATTG  
GTAACCCCTGAGACTAATATACACCTGTATGGGGTTTACTACAAAGGTTGGG  
AGGCCAAGGAACCTATCTGAGAGGACAGCAGGCAGAACTTATTCACATTGATA  
ATGTTTGAAGCCTTCGAGTTATTAGGGATGGACTCACAAGGGAAAAATAAGGAA  
CCTGTCCGGAAATTACATTTTGGATTGATATACGGCCTACACAAGCAAATCAA  
CAGAGGGCTGAAGAAAATGGTACTGGGGTGGGCCCTGCACCCTTTAGTTGTG  
ACTGGACCCCTAGTGACGAGAGGATCAGATTGCCAACAGACAACCTATTTGAGG  
GTAGAAACCAGGTGCCCATGTGGCTATGAGATGAAAGCTTTCAAAAATGTAGG  
TGGCAAACCTTACCAAAGTGGAGGAGCGGGCCTTTCCTATGTAGAAACAGAC  
CTGGTAGGGGACCAGTCAACTACAGAGTCAACCAAGTATTACGATGACAACCTC  
AGAGAGATAAAACCAGTAGCAAAGTTGGAAGGACAGGTAGAGCACTACTACAA  
AGGGGTCACAGCAAAAATTGACTACAGTAAAGGAAAAATGCTCTTGGCCACTG  
ACAAGTGGGAGGTGGAACATGGTGTCTATAACCAGGTTAGCTAAGAGATATACT  
GGGGTCGGGTTCAATGGTGCATACTTAGGTGACGAGCCCAATCACCGTGTCT  
AGTGGAGAGGGACTGTGCAACTATAACCAAAAACACAGTACAGTTTCTAAAAAT  
GAAGAAGGGGTGTGCGTTTACCTATGACCTGACCATCTCCAATCTGACCGGC  
TCATCGAACTAGTACACAGGAACAATCTTGAAGAGAAGGAAATACCCACCGCT  
ACGGTCACCATATGGCTAGCTTACACCTTCGTGAATGAAGACGTAGGGACTAT  
AAAACCAGTACTAGGAGAGAGAGTAATCCCCGACCCTGTAGTTGATATCAATTT  
ACAACCAGAGGTGCAAGTGGACACGTGAGAGGTTGGGATCACAATAATTGGAA  
GGGAAACCCCTGATGACAACGGGAGTGACACCTGTCTTGGAAAAAGTAGAGCCT  
GACGCCAGCGACAACCAAACTCGGTGAAGATCGGGTTGGATGAGGGTAATTA  
CCCAGGGCCTGGAATACAGACACATACACTAACAGAAAGAAATACACAACAGG  
ATGCGAGGCCCTTCATCATGATCCTGGGCTCAAGGAATTCCATATCAAAATAGGG  
CAAAGACTGTAGAAATATAAATCTGTACACAGGAAATGACCCCAGGGAAATA  
CGAGACTTGATGGCTGCAGGGCGCATGTTAGTAGTAGCACTGAGGGATGTCGA  
CCCTGAGCTGTCTGAAATGGTCGATTTCAAGGGGACTTTTTTAGATAGGGAGG  
CCCTGGAGGCTCTAAGTCTCGGGCAACCTAAACCGAAGCAGGTTACCAAGGAA  
GCTGTTAGGAATTTGATAGAACAGAAAAAGATGTGGAGATCCCTAACTGGTTT  
GCATCAGATGACCCAGTATTTCTGGAAGTGGCCTTAAAAAATGATAAGTACTAC  
TTAGTAGGAGATGTTGGAGAGCTAAAAGATCAAGCTAAAGCACTTGGGGCCAC  
GGATCAGACAAGAATTATAAAGGAGGTAGGCTCAAGGACGTATGCCATGAAGC  
TATCTAGCTGGTTCCTCAAGGCATCAAACAAACAGATGAGTTTAACTCCACTGT  
TTGAGGAATTGTTGCTACGGTGCCACCTGCAACTAAGAGCAATAAGGGGGCAC  
ATGGCATCAGCTTACCAATTGGCACAGGGTAACTGGGAGCCCCCTCGGTTGCGG  
GGTGACCTAGGTACAATACCAGCCAGAGGGGTGAAGATACACCCATATGAAG  
CTTACCTGAAGTTGAAAGATTTATAGAAGAAGAAGAGAAGAAACCTAGGGTT  
AAGGATACAGTAATAAGAGAGCACAAACAAATGGATACTTAAAAAATAAGGTTT  
CAAGGAAACCTCAACACCAAGAAAAATGCTCAACCCGGGGAACTATCTGAACA  
GTTGGACAGGGAGGGGCGCAAGAGGAACATCTACAACCACCAGATTGGTACT  
ATAATGTCAAGTGCAGGCATAAGGCTGGAGAAAATTGCCAATAGTGAGGGCCCA  
AACCGACACCAAAACCTTTCATGAGGCAATAAGAGATAAGATAGACAAGAGTG  
AAAACCGGCAAAATCCAGAATTGCACAACAAATTTGTTGGAGATTTCCACACGA  
TAGCCCAACCCACCCTGAAACACACCTACGGTGAGGTGACGTGGGAGCAACTT  
GAGGCGGGGATAAATAGAAAGGGGGCAGCAGGCTTCCTGGAGAAGAAGAACA  
TCGGAGAAGTATTGGATTCAGAAAAGCACCTGGTAGAACAATTGGTCAGGGAT  
CTGAAGGCCGGGAGAGAAAGATAAAATATTATGAACTGCAATACCAAAAAATGA  
GAAGAGAGATGTCAGTGATGACTGGCAGGCAGGGGACCTGGTGGTTGAGAGG  
AGGCCAAGAGTTATCCAATACCTGAAGCCAAGACAAGGCTAGCCATCACTAA  
GGTCATGTATAACTGGGTGAAACAGCAGCCCGTTGTGATTCCAGGATATGAAG  
GAAAGACCCCTTGTTCACATCTTTGATAAAGTGAGAAAGGAATGGGACTCGT  
TCAATGAGCCAGTGGCCGTAAAGTTTTGACACCAAGCCTGGGACACTCAAGTG  
ACTAGTAAGGATCTGCAACTTATTGGAGAAATCCAGAAATATTACTATAAGAAG  
GAGTGGCACAAGTTCATTGACACCATCACCGACCACATGACAGAAGTACCAGT

FIGURE 19-4

[illegible]

**FIGURE 19-5**

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	AATTCTGCTCATGACGGCCGTCGGCGTCAGCAGCTGAAGGTTGGGGTAAACACTCCGGCCCTCTTAGGCCA	
	10 20 30 40 50 60 70	
3H3Bfrag	AATTCTGCTCATGACGGCCGTCGGCGTCAGCAGCTGAAGGTTGGGGTAAACACTCCGGCCCTCTTAGGCCA	70
1.1.4 seq	AATTCTGCTCATGACGGCCGTCGGCGTCAGCAGCTGAAGGTTGGGGTAAACACTCCGGCCCTCTTAGGCCA	70
1.2.3 seq	AATTCTGCTCATGACGGCCGTCGGCGTCAGCAGCTGAAGGTTGGGGTAAACACTCCGGCCCTCTTAGGCCA	70
6.2.2 seq	AATTCTGCTCATGACGGCCGTCGGCGTCAGCAGCTGAAGGTTGGGGTAAACACTCCGGCCCTCTTAGGCCA	70
6.1.4 seq	AATTCTGCTCATGACGGCCGTCGGCGTCAGCAGCTGAAGGTTGGGGTAAACACTCCGGCCCTCTTAGGCCA	70
	TTTCCGTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT	
	80 90 100 110 120 130 140	
3H3Bfrag	TTTCCGTTT	140
1.1.4 seq	TTTCCGTTT	109
1.2.3 seq	TTTCCGTTT	102
6.2.2 seq	TTTCCGTTT	99
6.1.4 seq	TTTCCGTTT	84
	CTTTCCTCTTTTTTTTCTTTCTTTCTTCCTTCTTTAATG	
	150 160 170 180 190 200 210	
3H3Bfrag	TTTTTCTT	210
1.1.4 seq	TTTTTCTT	149
1.2.3 seq	TTTTTCTT	142
6.2.2 seq	TTTTTCTT	140
6.1.4 seq	TTTTTCTT	125
	GTGGCTCCATCTTAGCCCTAGTCACGGCTAGCTGTGAAAGGTCGGTGAGCCGCATGACTGCAGAGAGTGC	
	220 230 240 250 260 270 280	
3H3Bfrag	GTGGCTCCATCTTAGCCCTAGTCACGGCTAGCTGTGAAAGGTCGGTGAGCCGCATGACTGCAGAGAGTGC	280
1.1.4 seq	GTGGCTCCATCTTAGCCCTAGTCACGGCTAGCTGTGAAAGGTCGGTGAGCCGCATGACTGCAGAGAGTGC	219
1.2.3 seq	GTGGCTCCATCTTAGCCCTAGTCACGGCTAGCTGTGAAAGGTCGGTGAGCCGCATGACTGCAGAGAGTGC	212
6.2.2 seq	GTGGCTCCATCTTAGCCCTAGTCACGGCTAGCTGTGAAAGGTCGGTGAGCCGCATGACTGCAGAGAGTGC	210
6.1.4 seq	GTGGCTCCATCTTAGCCCTAGTCACGGCTAGCTGTGAAAGGTCGGTGAGCCGCATGACTGCAGAGAGTGC	195
	TGATACTGGCCTCTCTGCAGATCATGTCCCCCGGCCGTCGGCGTCAGCAGCTGAGACAAAATGTATATAT	
	290 300 310 320 330 340 350	
3H3Bfrag	TGATACTGGCCTCTCTGCAGATCATGTCCCCCGGCCGTCGGCGTCAGCAGCTGAGACAAAATGTATATAT	350
1.1.4 seq	TGATACTGGCCTCTCTGCAGATCATGTCCCCCGGCCGTCGGCGTCAGCAGCTGAGACAAAATGTATATAT	289
1.2.3 seq	TGATACTGGCCTCTCTGCAGATCATGTCCCCCGGCCGTCGGCGTCAGCAGCTGAGACAAAATGTATATAT	282
6.2.2 seq	TGATACTGGCCTCTCTGCAGATCATGTCCCCCGGCCGTCGGCGTCAGCAGCTGAGACAAAATGTATATAT	280
6.1.4 seq	TGATACTGGCCTCTCTGCAGATCATGTCCCCCGGCCGTCGGCGTCAGCAGCTGAGACAAAATGTATATAT	265
	TGTAAATAAATTAATCCATGTACATAGTGTATATAAATATAGTTGGGACCGT	
	360 370 380 390 400	
3H3Bfrag	TGTAAATAAATTAATCCATGTACATAGTGTATATAAATATAGTTGGGACCGT	402
1.1.4 seq	TGTAAATAAATTAATCCATGTACATAGTGTATATAAATATAGTTGGGACCGT	341
1.2.3 seq	TGTAAATAAATTAATCCATGTACATAGTGTATATAAATATAGTTGGGACCGT	334
6.2.2 seq	TGTAAATAAATTAATCCATGTACATAGTGTATATAAATATAGTTGGGACCGT	332
6.1.4 seq	TGTAAATAAATTAATCCATGTACATAGTGTATATAAATATAGTTGGGACCGT	317

FIGURE 20

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HCV/BVDV chimera

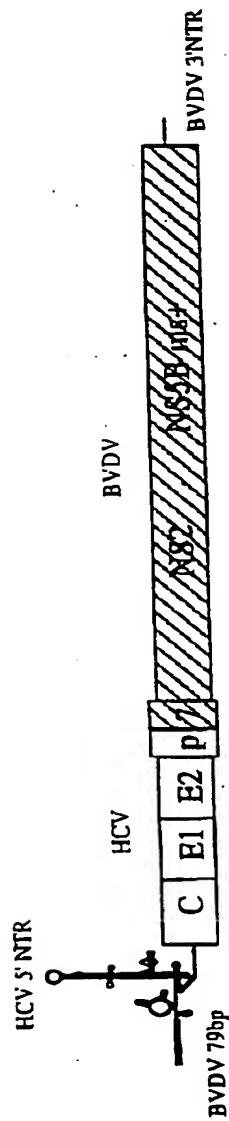


FIGURE 21

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Gtatacgagaattagaaaaggcactcgtatacgtattgggcaattaaataataattaggcctaggtacatggcacgtgccagccccc  
gatggggcgacactccaccatgaatcactcccctgtgaggaactactgtcttcacgcagaaagcgtctagccatggcgttagtatgag  
tgcgtgcagcctccaggacccccctccgggagagccatagtggtctgcggaaccggtgagtagaccggaattgccaggacgac  
cgggtccttcttgataaaccgctcaatgcctggagattggcggtgccccgcaagactgctagccgagtagtgggtcgcgaa  
aggccttgggtactgcctgatagggtgcttgcgagtgccccgggaggtctcgtagaccgtgcaccATGAGCACGAATC  
CTAAACCTCAAAGAAAAACCAAACGTAACACCAACCGTCGCCCCACAGGACGTC  
AAGTTCCCGGGTGGCGGTGATCGTTGGTGGAGTTTACTTGTGCGCGCAG  
GGGCCCTAGATTGGGTGTGCGCGCGACGAGGAAGACTTCCGAGCGGTGCGCAA  
CCTCGAGGTAGACGTCAGCCTATCCCCAAGGCACGTCGGCCCCGAGGGCAGGA  
CTGGGGCTCAGCCCCGGGTACCCTTGGCCCCCTCTATGGCAATGAGGGTTGCGGG  
TGGCGGGGATGGCTCCTGTCTCCCCGTGGCTCTCGGCCTAGCTGGGGCCCCAC  
AGACCCCCGGCGTAGGTCGCGCAATTTGGGTAAAGGTCATCGATACCCCTTACGT  
GCGGCTTCGCGGACCTCATGGGGTACATACCGCTCGTCGGCGCCCCCTCTTGGA  
GGCGCTGCCAGGGCCCTGGCGCATGGCGTCCGGGTTCTGGAAGACGGCGTGA  
ACTATGCAACAGGGAACCTTCTGCTTCTCTCTATCTTCTTCTGGCCCT  
GCTCTCTTGCCTGACCGTGCCCGCTTCAGCCTACCAAGTGCGCAATTCCTCGGG  
GCTTTACCATGTCACCAATGATTGCCCTAACTCGAGTATTGTGTACGAGGCGGC  
CGATGCCATCCTGCACACTCCGGGGTGTGTCCCTTGC GTTCCGAGGGTAAACG  
CCTCGAGGTGTTGGGTGGCGGTGACCCCCACGGTGGCCACCAGGGACGGCAA  
ACTCCCCACAACGCAGCTTCGACGTCATATCGATCTGCTTGTGCGGGAGCGCCA  
CCCTCTGCTCGGCCCTCTACGTGGGGGACCTGTGCGGGTCTGTCTTCTTGTG  
GTCAACTGTTTACCTTCTCTCCAGGCGCCACTGGACGACGCAAGACTGCAATT  
GTTCTATCTATCCCGGCCATATAACGGGTGTCATCGCATGGCATGGGATATGATGA  
TGAACGTGGTCCCCTACGGCAGCGTTGGTGGTAGCTCAGTGTCTCCGATCCCA  
CAAGCCATCATGGACATGATCGTGGTGTCTACTGGGGAGTCCCTGGCGGCGCAT  
AGCGTATTTCTCCATGGTGGGGAACCTGGGCGAAGGTCCTGGTAGTGCTGCTGC  
TATTTGCCGGCGTCGACGCGGAAACCCACGTCACCGGGGGAAGTGCCGGCCG  
CACCACGGCTGGGCTTGTGGTCTCCTTACACCAGGCGCCAAGCAGAACATCC  
AACTGATCAACACCAACGGCAGTTGGCACATCAATAGCACGGCCTTGAACGTC  
AATGAAAGCCTTAACACCGGCTGGTTAGCAGGGCTCTTCTATCAGCACAAATTC  
AACTCTTCAGGCTGTCTGAGAGGTTGGCCAGTGGCCGACGCTTACCGATTTT  
GCCCCAGGCTGGGGTCTATCAGTTATGCCAACGGAAGCGGCCTCGACGAAC  
GCCCCCTACTGCTGGCACTACCCTCCAAGACCTTGTGGCATTGTGCCCCGAAAG  
AGCGTGTGTGGCCCGGTATATTGCTTCACTCCAGCCCCGTGGTGGTGGGAAC  
GACCGACAGGTCGGGCGCGCCTACCTACAGCTGGGGTGCAAATGATACGGAT  
GTCTTCGTCTTAACAACACCAAGGCCACCGCTGGGCAATTGGTTCCGTTGTACC  
TGGATGAACTCAACTGGATTACCAAAAGTGTGCGGAGCGCCCCCTTGTGTCTAT  
CGGAGGGGTGGGCAACAACACCTTGTCTGCCCCACTGATTGTTTCCGCAAGC  
ATCCGGAAGCCACATACTCTCGGTGCGGCTCCGGTCCCTGGATTACACCCAGG  
TGCATGGTCGACTACCCGTATAGGCTTTGGCACTATCCTTGTACCATCAATTAC  
ACCATATTCAAAGTCAGGATGTACGTGGGAGGGGTGAGCAGACAGGCTGGAAG  
CGGCCTGCAACTGGACGCGGGGCGAACGCTGTGATCTGGAAGACAGGGACAG  
GTCCGAGCTCAGCCCCATTGCTGCTGTGCCACACAGTGGCAGGTCCTTCCGT  
GTTCTTTACGACCCCTGCCAGCCTTGTCCACCGGCCTCATCCACCTCCACCAGA  
ACATTGTGGACGTGCAGTACTTGTACGGGGTAGGGTCAAGCATCGCGTCTTGG  
GCCATTAAGTGGGAGTACGTGCTTCTCCTGTTCTCCTGCTTGCAGACGCGCGC  
GTCTGCTCCTGCTTGTGGATGATGTTACTCATATCCCAAGCGGAGGCGGCTTTG  
GAGAACCTCGTAATACTCAATGCAGCATCCCTGGCCGGGACGCACGGTCTTGT  
GTCCTTCTCGTGTCTTCTGCTTTGCGTGGTATCTGAAGGGTAGGTGGGTGCC

FIGURE 22-1

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CGGAGCGGTCTACGCCTTCTACGGGAAGTGGGTCTTACTCTTATACCACATCTT  
AGTGGTACACCCAATCAAATCTGTAATTGTGATCCTACTGATGATTGGGGATGT  
GGTAAAGGCCGATTACAGGGGGCCAAGAGTACTTGGGGAAAATAGACCTCTGTT  
TTACAACAGTAGTACTAATCGTCATAGGTTTAAATCATAGCTAGGCGTGACCCAA  
CTATAGTGCCACTGGTAACAATAATGGCAGCACTGAGGGTCACTGAACTGACC  
CACCAGCCTGGAGTTGACATCGCTGTGGCGGTCTGACTATAACCCTACTGAT  
GGTTAGCTATGTGACAGATTATTTTAGATATAAAAAATGGTTACAGTGCATTCTC  
AGCCTGGTATCTGCGGTGTTCTTGATAAGAAGCCTAATATACCTAGGTAGAATC  
GAGATGCCAGAGGTAACCTATCCCAAATGGAGACCACTAATTTAATACTATTA  
TATTTGATCTCAACAACAATTGTAACGAGGTGGAAGGTTGACGTGGCTGGCCTA  
TTGTTGCAATGTGTGCCTATCTTATTGCTGGTCAACAACCTTGTGGGCCGACTTCT  
TAACCCTAATACTGATCCTGCCTACCTATGAATTGGTTAAATTATACTATCTGAA  
AACTGTTAGGACTGATACAGAAAGAAGTTGGCTAGGGGGGATAGACTATACAA  
GAGTTGACTCCATCTACGACGTTGATGAGAGTGGAGAGGGCGTATATCTTTTTTC  
CATCAAGGCAGAAAGCACAGGGGAATTTTTCTATACTCTTGCCCTTATCAAAG  
CAACACTGATAAGTTGCGTCAGCAGTAAATGGCAGCTAATATACATGAGTTACT  
TAACCTTTGGACTTTATGTACTACATGCACAGGAAAGTTATAGAAGAGATCTCAG  
GAGGTACCAACATAATATCCAGGTTAGTGGCAGCACTCATAGAGCTGAACTGG  
TCCATGGAAGAAGAGGAGAGCAAAGGCTTAAAGAAGTTTTATCTATTGTCTGG  
AAGGTTGAGAAACCTAATAATAAAACATAAGGTAAGGAATGAGACCGTGGCTT  
CTTGGTACGGGGAGGAGGAAGTCTACGGTATGCCAAAGATCATGACTATAATC  
AAGGCCAGTACACTGAGTAAGAGCAGGCACTGCATAATATGCACCTGTATGTGA  
GGCCCGAGAGTGGAAAGGTGGCAGCTGCCCAAATGTGGACGCCATGGGAAG  
CCGATAACGTGTGGGATGTGCTAGCAGATTTTGAAGAAAGACACTATAAAAG  
AATCTTTATAAGGGAAGGCAACTTTGAGGGTATGTGCAGCCGATGCCAGGGAA  
AGCATAGGAGGTTTGAAATGGACCGGGAACCTAAGAGTGCCAGATACTGTGCT  
GAGTGTAAATAGGCTGCATCCTGCTGAGGAAGGTGACTTTTGGGCAGAGTCCGAG  
CATGTTGGGCCCTCAAAATCACCTACTTTGCGCTGATGGATGGAAAGGTGTATGA  
TATCACAGAGTGGGCTGGATGCCAGCTGTGGGAATCTCCCCAGATACCCACA  
GAGTCCCTTGTACATCTCATTTGGTTACGGATGCCTTTCAGGCAGGAATACA  
ATGGCTTTGTACAATATACCGCTAGGGGGCAACTATTTCTGAGAACTTGCCCG  
TACTGGCAACTAAAGTAAAAATGCTCATGGTAGGCAACCTTGGAGAAGAAATT  
GGTAATCTGGAACATCTTGGGTGGATCCTAAGGGGGCCTGCCGTGTGTAAGAA  
GATCACAGAGCACGAAAAATGCCACATTAATATACTGGATAAACTAACCGCATT  
TTTCGGGATCATGCCAAGGGGGACTACACCCAGAGCCCCGGTGAGGTTCCCTA  
CGAGCTTACTAAAAGTGAGGAGGGGTCTGGAGACTGCCTGGGCTTACACACAC  
CAAGGCGGGATAAGTTCAGTCGACCATGTAACCGCCGGAAAAGATCTACTGGT  
CTGTGACAGCATGGGACGAACTAGAGTGGTTTGCCAAAGCAACAACAGGTTGA  
CCGATGAGACAGAGTATGGCGTCAAGACTGACTCAGGGTGCCAGACGGTGC  
CAGATGTTATGTGTTAAATCCAGAGGCCGTTAACATATCAGGATCCAAAGGGG  
CAGTCGTTACCTCCAAAAGACAGGTGGAGAATTCACGTGTGTACCGCATCA  
GGCACACCGGCTTTCTTCGACCTAAAAAACTTGAAAGGATGGTCAGGCTTGCCCT  
ATATTTGAAGCCTCCAGCGGGAGGGTGGTTGGCAGAGTCAAAGTAGGGAAGA  
ATGAAGAGTCTAAACCTACAAAAATAATGAGTGGAATCCAGACCGTCTCAAAAA  
ACAGAGCAGACCTGACCGAGATGGTCAAGAAGATAACCAGCATGAACAGGGG  
AGACTTCAAGCAGATTACTTTGGCAACAGGGGCAGGCAAAACCACAGAACTCC  
CAAAAGCAGTTATAGAGGAGATAGGAAGACACAAGAGAGTATTAGTTCTTATA  
CCATTAAGGGCAGCGGCAGAGTCAGTCTACCAGTATATGAGATTGAACACACC  
AAGCATCTCTTTTAACCTAAGGATAGGGGACATGAAAGAGGGGGACATGGCAA  
CCGGGATAACCTATGCATCATACGGGTACTTCTGCCAAATGCCTCAACCAAAGC  
TCAGAGCTGCTATGGTAGAATACTCATACATATTCTTAGATGAATACCATTGTGC  
CACTCCTGAACAACTGGCAATTATCGGGAAGATCCACAGATTTTCAGAGAGTAT  
AAGGGTTGTGCGCCATGACTGCCACGCCAGCAGGGTCGGTGACCACAACAGGT  
CAAAAGCACCCAATAGAGGAATTCATAGCCCCGAGGTAATGAAAGGGGAGG

FIGURE 22-2

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ATCTTGGTAGTCAGTTCCTTGATATAGCAGGGTTAAAAATACCAGTGGATGAGA  
TGAAAGGCAATATGTTGGTTTTTGTACCAACGAGAAACATGGCAGTAGAGGTA  
GCAAAGAAGCTAAAAGCTAAGGGCTATAACTCTGGATACTATTACAGTGGAGA  
GGATCCAGCCAATCTGAGAGTTGTGACATCACAAATCCCCCTATGTAATCGTGGC  
TACAAATGCTATTGAATCAGGAGTGACACTACCAGATTTGGACACGGTTATAGA  
CACGGGGTTGAAATGTGAAAAGAGGGTGAGGGTATCATCAAAGATACCCTTCA  
TCGTAACAGGCCTTAAGAGGATGGCCGTGACTGTGGGTGAGCAGGCGCAGCG  
TAGGGGCAGAGTAGGTAGAGTGAAACCCGGGAGGTATTATAGGAGCCAGGAA  
ACAGCAACAGGGTCAAAGGACTACCCTATGACCTCTTGACAGGCACAAAGATA  
CGGGATTGAGGATGGAATCAACGTGACGAAATCCTTTAGGGAGATGAATTACG  
ATTGGAGCCTATACGAGGAGGACAGCCTACTAATAACCCAGCTGGAAATACTA  
AATAATCTACTCATCTCAGAAGACTTGCCAGCCGCTGTAAAGAACATAATGGCC  
AGGACTGATCACCCAGAGCCAATCCAACCTTGATACAAACAGCTATGAAGTCCA  
GGTCCCGGTCTATTCCCAAAAATAAGGAATGGAGAAGTCACAGACACCTACG  
AAAATTACTCGTTTCTAAATGCCAGAAAGTTAGGGGAGGATGTGCCCGTGTATA  
TCTACGCTACTGAAGATGAGGATCTGGCAGTTGACCTCTTAGGGCTAGACTGG  
CCTGATCCTGGGAACCAGCAGGTAGTGGAGACTGGTAAAGCACTGAAGCAAGT  
GACCGGGTTGTCCTCGGCTGAAAATGCCCTACTAGTGGCTTTATTTGGGTATGT  
GGGTTACCAGGCTCTCTCAAAGAGGCATGTCCCAATGATAACAGACATATATAC  
CATCGAGGACCAGAGACTAGAAGACACCACCCACCTCCAGTATGCACCCAACG  
CCATAAAAACCGATGGGACAGAGACTGAACTGAAAGAACTGGCGTCCGGGTGA  
CGTGGAATAATCATGGGAGCCATTTAGATTATGCAGCTGGGGGACTGGAGT  
TTGTTAAATCCCAAGCAGAAAAGATAAAAACAGCTCCTTTGTTTAAAGAAAACG  
CAGAAGCCGCAAAAAGGGTATGTCCAAAATTCATTGACTCATTAAATTGAAAATA  
AAGAAGAAATAATCAGATATGGTTTGTGGGGAACACACACAGCACTATACAAA  
AGCATAGCTGCAAGACTGGGGCATGAAACAGCGTTTGCCACACTAGTGTAAA  
GTGGCTAGCTTTTGGAGGGGAATCAGTGTGACACCACGTCAAGCAGGCGGCA  
GTTGATTTAGTGGTCTATTATGTGATGAATAAGCCTTCCTTCCCAGGTGACTCC  
GAGACACAGCAAGAAGGGAGGCGATTCTGCGCAAGCCTGTTTATCTCCGCACT  
GGCAACCTACACATACAAAACCTTGGAATTACCAATCTCTCTAAAGTGGTGA  
ACCAGCCCTGGCTTACCTCCCCTATGCTACCAGCGCATTAAAAATGTTACCCCC  
AACGCGGCTGGAGAGCGTGGTGATACTGAGCACCACGATATATAAACATACC  
TCTCTATAAGGAAGGGGAAGAGTGATGGATTGCTGGGTACGGGGATAAGTGC  
AGCCATGGAAATCCTGTACAAAACCCAGTATCGGTAGGTATATCTGTGATGTT  
GGGGGTAGGGGCAATCGCTGCGCACAAACGCTATTGAGTCCAGTGAACAGAAA  
AGGACCCTACTTATGAAGGTGTTGTAAAGAACTTCTTGGATCAGGCTGCAACA  
GATGAGCTGGTAAAAGAAAACCCAGAAAAAATTATAATGGCCTTATTTGAAGCA  
GTCCAGACAATTGGTAACCCCTGAGACTAATATACCACCTGTATGGGGTTTAC  
TACAAAGGTTGGGAGGCCAAGGAACTATCTGAGAGGACAGCAGGCAGAAACT  
TATTCACATTGATAATGTTTGAAGCCTTCGAGTTATTAGGGATGGACTCACAAG  
GGAAAATAAGGAACCTGTCCGGAATTAATTTTGGATTTGATATACGGCCTAC  
ACAAGCAAAATCAACAGAGGGCTGAAGAAAATGGTACTGGGGTGGGCCCTGC  
ACCCTTTAGTTGTGACTGGACCCCTAGTGACGAGAGGATCAGATTGCCAACAG  
ACAACTATTTGAGGGTAGAAACCAGGTGCCCATGTGGCTATGAGATGAAAGCT  
TTCAAAAATGTAGGTGGCAAACTTACCAAAGTGGAGGAGAGCGGGCCTTTCT  
ATGTAGAAACAGACCTGGTAGGGGACCAGTCAACTACAGAGTCACCAAGTATT  
ACGATGACAACCTCAGAGAGATAAAACCAAGTAGCAAAGTTGGAAGGACAGGTA  
GAGCACTACTACAAAGGGTCAAGCAAAAAATTGACTACAGTAAAGGAAAAAT  
GCTCTTGGCCACTGACAAAGTGGGAGGTGGAACATGGTGTCAATACAGGTTAG  
CTAAGAGATATACTGGGGTCCGGTTCAATGGTGCATACTTAGGTGACGAGCCC  
AATCACCGTGCTCTAGTGGAGAGGGACTGTGCAACTATAACCAAAAACACAGT  
ACAGTTTCTAAAAATGAAGAAGGGGTGTGCGTTACCTATGACCTGACCATCTC  
CAATCTGACCAGGCTCATCGAACTAGTACACAGGAACAATCTTGAAGAGAAGG  
AAATACCCACCGCTACGGTCACCACATGGCTAGCTTACACCTTCGTGAATGAAG

FIGURE 22-3



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ACGTAGGGACTATAAAACCACTAGGAGAGAGAGTAATCCCCGACCCTGTA  
GTTGATATCAATTTACAACCAGAGGTGCAAGTGGACACGTCAGAGGTTGGGAT  
CACAATAATTGGAAGGGAAACCCTGATGACAACGGGAGTGACACCTGTCTTG  
AAAAAGTAGAGCCTGACGCCAGCGACAACCAAACTCGGTGAAGATCGGGTTG  
GATGAGGGTAATTACCCAGGGCCTGGAATACAGACACATACTAACAGAAGA  
AATACACAACAGGGATGCGAGGGCCCTTCATCATGATCCTGGGCTCAAGGAATT  
CCATATCAAATAGGGCAAAGACTGCTAGAAATATAAATCTGTACACAGGAAATG  
ACCCAGGGAAATACGAGACTTGATGGTGCAGGGCGCATGTTAGTAGTAGCA  
CTGAGGGATGTCGACCCTGAGCTGTCTGAAATGGTCGATTTCAAGGGGACTTT  
TTTAGATAGGGAGGGCCTGGAGGCTCTAAGTCTCGGGCAACCTAAACCGAAGC  
AGGTTACCAAGGAAGCTGTTAGGAATTTGATAGAACAGAAAAAGATGTGGAG  
ATCCCTAACTGGTTTGCATCAGATGACCCAGTATTTCTGGAAGTGGCCTTAAAA  
AATGATAAGTACTACTTAGTAGGAGATGTTGGAGAGCTAAAAGATCAAGCTAAA  
GCACTTGGGGGCCACGGATCAGACAAGAATTATAAAGGAGGTAGGCTCAAGGA  
CGTATGCCATGAAGCTATCTAGCTGGTTCCTCAAGGCATCAAACAAACAGATGA  
GTTTAACTCCACTGTTTGAGGAATTGTTGCTACGGTGCCACCTGCAACTAAGA  
GCAATAAGGGGCACATGGCATCAGCTTACCAATTGGCACAGGGTAACTGGGAG  
CCCCTCGGTTGCGGGGTGCACCTAGGTACAATACCAGCCAGAAGGGTGAAGAT  
ACACCCATATGAAGCTTACCTGAAGTTGAAAGATTTTCATAGAAGAAGAAGAGAA  
GAAACCTAGGGTTAAGGATACAGTAATAAGAGAGCACAACAAATGGATACTTA  
AAAAAATAAGGTTTCAAGGAAACCTCAACACCAAGAAAAATGCTCAACCCAGGG  
AACTATCTGAACAGTTGGACAGGGAGGGCGCAAGAGGAGTCACTCAACACCA  
CCAGATTGGTACTATAATGTCAAGTGCAGGCATAAGGCTGGAGAAATTGCCAA  
TAGTGAGGGCCCCAAACCGACACCAAAACCTTTCATGAGGCAATAAGAGATAAG  
ATAGACAAGAGTGAAAACCGGCAAAATCCAGAATTGCACAACAAATTGTTGGA  
GATTTTCCACACGATAGCCCAACCCACCTGAAACACACCTACGGTGAGGTGA  
CGTGGGAGCAACTTGAGGCGGGGGTAAATAGAAAGGGGGCAGCAGGCTTCCT  
GGAGAAGAAGAACATCGGAGAAGTATTGGATTTCAGAAAAGCACCTGGTAGAAC  
AATTGGTCAGGGATCTGAAGGCCGGGAGAAAGATAAAATATTATGAACTGCA  
ATACCAAAAAATGAGAAGAGAGATGTCAAGTGCAGTGCAGTGGCAGGCAGGGGACC  
TGGTGGTTGAGAAGAGGGCCAAGAGTTATCCAATACCCTGAAGCCAAGACAAGG  
CTAGCCATCACTAAGGTCATGTATAACTGGGTGAAACAGCAGCCCGTTGTGATT  
CCAGGATATGAAGGAAAGACCCCTTGTTCACATCTTTGATAAAGTGAAGAAAG  
GAATGGGACTCGTTCAATGAGCCAGTGCCCGTAAGTTTGGACACCAAGCCTG  
GGCACTCAAGTGACTAGTAAGGATCTGCAACTTATTGGAGAAATCCAGAAATA  
TTACTATAAGAAGGAGTGGCACAAGTTTATTGACACCATCACCGACCACATGAC  
AGAAGTACCAGTTATAACAGCAGATGGTGAAGTATATATAAGAAATGGGCAGA  
GAGGGAGCGGCCAGCCAGACACAAGTGCTGGCAACAGCATGTTAAATGTCCT  
GACAATGATGTACGGCTTCTGCGAAAGCACAGGGGTACCGTACAAGAGTTTCA  
ACAGGGTGGCAAGGATCCACGTCTGTGGGGATGATGGCTTCTTAATAACTGAA  
AAAGGGTTAGGGCTGAAATTTGCTAACAAAGGGATGCAGATTCTTCATGAAGC  
AGGCAAAACCTCAGAAGATAACGGAAGGGGAAAAGATGAAAGTTGCCTATAGAT  
TTGAGGATATAGAGTTCTGTTCTCATACCCAGTCCCTGTTAGGTGGTCCGACA  
ACACCAGTAGTCACATGGCCGGGAGAGACACCGCTGTGATACTATCAAAGATG  
GCAACAAGATTGGATTCAAGTGGAGAGAGGGGTACCACAGCATATGAAAAAGC  
GGTAGCCTTCAGTTTCTTGCTGATGTATTCCTGGAACCCGCTTGTAGGAGGAT  
TTGCCTGTTGGTCTTTTCGAAACAGCCAGACAGACCCATCAAACATGCCAC  
TTATTATTACAAAGGTGATCCAATAGGGGCTATAAAGATGTAATAGGTGCGAA  
TCTAAGTGAAGTGAAGAGAACAGGCTTTGAGAAATTGGCAAATCTAAACCTAAG  
CCTGTCCACGTTGGGGGTCTGGACTAAGCACACAAGCAAAAGAATAATTCAGG  
ACTGTGTTGCCATTGGGAAAGAAGAGGGCAACTGGCTAGTTAAGCCCGACAGG  
CTGATATCCAGCAAACTGGCCACTTATACATACCTGATAAAGGCTTTACATTAC  
AAGGAAAGCATTATGAGCAACTGCAGCTAAGAACAGAGACAAACCCGGTCTG  
GGGTTGGGACTGAGAGATACAAGTTAGGTCCCATAGTCAATCTGCTGCTGAG

FIGURE 22-4

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AAGGTTGAAAATTCTGCTCATGACGGCCGTCGGCGTCAGCAGCTGAgacaaaatgtat  
atattgtaaataaattaatccatgtacatagtgtatataaatatagttgggaccgtccacctaagaagacgacacgccaacacgcacag  
ctaaacagtagtcaagattatctacctcaagataacactacattaatgcacacagcactttagctgtatgaggatacgcccgacgtctatag  
ttggactaggggaagacctctaacagcccc

FIGURE 22-5

HCV/BVDV chimera with selectable marker

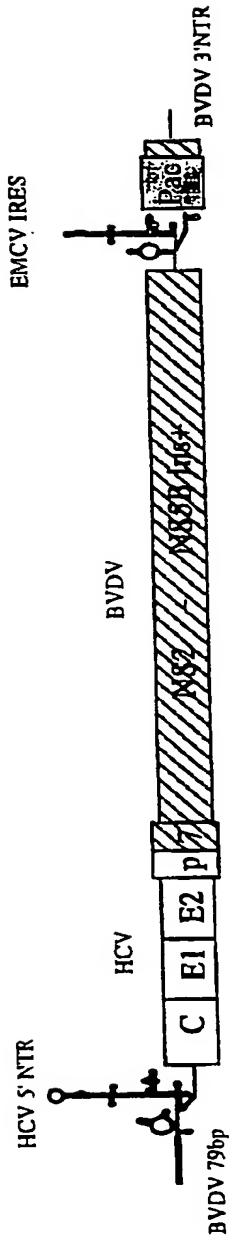


FIGURE 23

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Gtatacgagaattagaaaaggcactcgtatagcattgggcaataaaaaataaattaggcctaggtacatggcagtgccagccccct  
gatgggggacactccaccatgaatcactcccctgtgaggaactactgtcttcacgcagaaagcgtctagccatggcgttagtatgag  
tgtcgtgcagcctccaggacccccctcccgggagagccatagtgtctgcggaaccggtgagtacaccggaattgccaggacgac  
cgggtcccttcttgataaaccgctcaatgcctggagattggcggtgccccgcaagactgctagccgagtagtgggtcgga  
aggcctgtgtgactgctgatagggtgcttgcgagtgccccgggaggtctcgtagaccgtgcaccATGAGCACGAATC  
CTAAACCTCAAAGAAAAACCAAACGTAAACACCAACCGTCGCCCACAGGACGTC  
AAGTTCCTCCGGGTGGCGGTGAGATCGTTGGTGGAGTTTACTTGTGTCGCGCAG  
GGGCCCTAGATTGGGTGTGCGCGCGACGAGGAAGACTTCCGAGCGGTGCGAA  
CCTCGAGGTAGACGTCAGCCTATCCCCAAGGCACGTCGGCCCCGAGGGCAGGA  
CCTGGGCTCAGCCCCGGGTACCCCTTGCCCCCTCTATGGCAATGAGGGTTGCGGG  
TGGGCGGGTAGGGTCTGTCTCCCCGTGGCTCTCGGCCTAGCTGGGGCCCCAC  
AGACCCCCGGCGTAGGTGCGCAATTTGGGTAAAGGTCATCGATACCCCTTACGT  
GCGGCTTCGCGGACCTCATGGGGTACATACCGCTCGTCGGCGCCCCCTCTTGGA  
GGCGCTGCCAGGGCCCTGGCGCATGGCGTCCGGGTCTGGAAGACGGCGTGA  
ACTATGCAACAGGGAACCTTCCTGGTTGCTCTTTCTCTATCTTCTTCTGGCCCT  
GCTCTCTTGCCTGACCGTGCCCCGCTTACGCTACCAAGTGCGCAATTCCTCGGG  
GCTTTACCATGTACCAATGATTGGCTTCACTCGAGTATTGTGTACGAGGCGGC  
CGATGCCATCCTGCACACTCCGGGGTGTGTCCCTTGCCTTCGCGAGGGTAACG  
CCTCGAGGTGTTGGGTGGCGGTGACCCCCACGGTGGCCACCAGGGACGGCAA  
ACTCCCCACAACGCAGCTTCGACGTATATCGATCTGCTTGTGCGGGAGCGCCA  
CCCTCTGCTCGGCCCTCTACGTGGGGGACCTGTGCGGGTCTGTCTTTCTTGTG  
GTCAACTGTTTACCTTCTCTCCAGGGCCACTGGACGACGCAAGACTGCAATT  
GTTCTATCTATCCCGGCCATATAACGGGTCATCGCATGGCATGGGATATGATGA  
TGAAGTGTCCCTACGGCAGCGTTGGTGGTAGCTCAGCTGCTCCGGATCCCA  
CAAGCCATCATGGACATGATCGCTGGTGTCACTGGGGAGTCCTGGCGGGCAT  
AGCGTATTTCTCCATGGTGGGGAAGTGGGCGAAGGTCCTGGTAGTGTCTGCTGC  
TATTTGCCGGCGTCGACGCGGAAACCCACGTCACCGGGGGAAGTGCCGGGCC  
CACCACGGCTGGGCTTGTGGTCTCCTTACACCAGGCGCCAAGCAGAACATCC  
AACTGATCAACACCAACGGCAGTTGGCACATCAATAGCACGGCCTTGAAGTGC  
AATGAAAGCCTTAACACCGGCTGGTTAGCAGGGCTCTTCTATCAGCACAAATTC  
AACTCTTCAGGCTGTCTGAGAGGTTGGCCAGCTGCCGACGCCTTACCGATTIT  
GCCCAGGGCTGGGGTCTATCAGTTATGCCAACGGAAGCGGCCTCGACGAAC  
GCCCCTACTGCTGGCACTACCCCTCCAAGACCTTGTGGCATTGTGCCCGCAAAG  
AGCGTGTGTGGCCCCGGTATATTGCTTCACTCCCAGCCCCGTGGTGGTGGGAAC  
GACCGACAGGTCGGGCGCGCCTACCTACAGCTGGGGTGCAATGATACGGAT  
GTCTTCGTCCTTAACAACACACAGGCCACCGCTGGGCAATTGGTTCCGTTGTACC  
TGGATGAACTCAACTGGATTACCAAAGTGTGCGGAGCGCCCCCTTGTGTCTAT  
CGGAGGGGTGGGCAACAACACCTTGTCTGCCCCACTGATTGTTTCCGCAAGC  
ATCCGGAAGCCACATACTCTCGGTGCGGCTCCGGTCCCTGGATTACACCCAGG  
TGCATGGTCTGACTACCCGTATAGGCTTTGGCACTATCCTTGTACCATCAATTAC  
ACCATATTCAAAGTCAGGATGTACGTGGGAGGGTTCGAGCACAGGCTGGAAG  
CGGCCTGCAACTGGACGCGGGGCGAAGCGTGTGATCTGGAAGACAGGGACAG  
GTCCGAGCTCAGCCCATTGCTGCTGTCCACCACACAGTGGCAGGTCTTCCGT  
GTTCTTTACGACCCTGCCAGCCTTGTCCACCGGCCTCATCCACCTCCACCAGA  
ACATTGTGGACGTGCAGTACTTGTACGGGGTAGGGTCAAGCATCGCGTCTCTGG  
GCCATTAAGTGGGAGTACGTCGTTCTCCTGTTCTCCTGCTTGCAGACGCGCGC  
GTCTGCTCCTGCTTGTGGATGATGTTACTCATATCCCAAGCGGAGGCGGCTTTG  
GAGAACCTCGTAATACTCAATGCAGCATCCCTGGCCGGGACGCACGGTCTTGT

FIGURE 24-1

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GTCCTTCCTCGTGTCTCTCTGCTTTGCGTGGTATCTGAAGGGTAGGTGGGTGCC  
CGGAGCGGTCTACGCCTTCTACGGGAAGTGGGTCTTACTCTTATACCACATCTT  
AGTGGTACACCCAATCAAATCTGTAATTGTGATCCTACTGATGATTGGGGATGT  
GGTAAAGGCCGATTACAGGGGGCCAAGAGTACTTGGGGAAAATAGACCTCTGTT  
TTACAACAGTAGTACTAATCGTCATAGGTTTAAATCATAGCTAGGCGTGACCCAA  
CTATAGTGCCACTGGTAACAATAATGGCAGCACTGAGGGTCACTGAACCTGACC  
CACCAGCCTGGAGTTGACATCGCTGTGGCGGTCTATGACTATAACCCTACTGAT  
GGTTAGCTATGTGACAGATTATTTTAGATATAAAAAATGGTTACAGTGCAATCTC  
AGCCTGGTATCTGCGGTGTTCTTGATAAGAAGCCTAATATACCTAGGTAGAATC  
GAGATGCCAGAGGTAACCTATCCCAAATGGAGACCACTAACTTTAATACTATTA  
TATTTGATCTCAACAACAATTGTAACGAGGTGGAAGGTTGACGTGGCTGGCCTA  
TTGTTGCAATGTGTGCCTATCTTATTGCTGGTCACAACCTTGTGGGCCGACTTCT  
TAACCCTAATACTGATCCTGCCTACCTATGAATTGGTTAAATTATACTATCTGAA  
AACTGTTAGGACTGATACAGAAAGAAGTTGGCTAGGGGGGATAGACTATACAA  
GAGTTGACTCCATCTACGACGTTGATGAGAGTGGAGAGGGCGTATATCTTTTTTC  
CATCAAGGCAGAAAGCACAGGGGAATTTTCTATACTCTTGCCCCCTTATCAAAG  
CAACACTGATAAGTTGCGTCAGCAGTAAATGGCAGCTAATATACATGAGTTACT  
TAACTTTGGACTTTATGTACTACATGCACAGGAAAGTTATAGAAGAGATCTCAG  
GAGGTACCAACATAATATCCAGGTTAGTGGCAGCACTCATAGAGCTGAACCTGG  
TCCATGGAAGAAGAGGAGAGCAAAGGCTTAAAGAAGTTTATCTATTGTCTGG  
AAGGTTGAGAAACCTAATAATAAACATAAGGTAAGGAATGAGACCGTGGCTT  
CTTGGTACGGGGAGGAGGAAGTCTACGGTATGCCAAAGATCATGACTATAATC  
AAGGCCAGTACACTGAGTAAGAGCAGGCACTGCATAATATGCATGTATGTGA  
GGGCCGAGAGTGGAAAGGTGGCACCTGCCCAAATGTGGACGCCATGGGAA  
CCGATAACGTGTGGGATGTGCTAGCAGATTTTGAAGAAAGACACTATAAAAG  
AATCTTTATAAGGGAAGGCAACTTTGAGGGTATGTGCAGCCGATGCCAGGGAA  
AGCATAGGAGGTTTGAATGGACCGGGAACCTAAGAGTGCCAGATACTGTGCT  
GAGTGTAATAGGCTGCATCCTGCTGAGGAAGGTGACTTTTGGGCAGAGTTCGAG  
CATGTTGGGCCTCAAAATCACCTACTTTGCGCTGATGGATGGAAAGGTGTATGA  
TATCACAGAGTGGGCTGGATGCCAGCGTGTGGGAATCTCCCCAGATACCCACA  
GAGTCCCTTGTACATCTCATTTGGTTACGGATGCCTTTCAGGCAGGAATACA  
ATGGCTTTGTACAATATACCGCTAGGGGGCAACTATTTCTGAGAACTTGCCCG  
TACTGGCAACTAAAGTAAAAATGCTCATGGTAGGCAACCTTGGAGAAGAAATT  
GGTAATCTGGAACATCTTGGGTGGATCCTAAGGGGGCCTGCCGTGTGTAAGAA  
GATCACAGAGCACGAAAAATGCCACATTAATATACTGGATAAACTAACCGCATTT  
TTTCGGGATCATGCCAAGGGGGGACTACACCCAGAGCCCCGGTGAGGTTCCCTA  
CGAGCTTACTAAAAGTGAGGAGGGGTCTGGGAGACTGCCTGGGCTTACACACAC  
CAAGGCGGGATAAGTTCAGTCGACCATGTAACCGCCGGAAAGATCTACTGGT  
CTGTGACAGCATGGGACGAACTAGAGTGGTTTGCCAAAGCAACAACAGGTTGA  
CCGATGAGACAGAGTATGGCGTCAAGACTGACTCAGGGTGCCAGACGGTGC  
CAGATGTTATGTGTTAAATCCAGAGGCCGTTAACATATCAGGATCCAAAGGGG  
CAGTCGTTACCTCCAAAAGACAGGTGGAGAATTACGTTGTGCACCGCATCA  
GGCACACCGGCTTTCTTCGACCTAAAAAACTTGAAAGGATGGTCAGGCTTGCT  
ATATTTGAAGCCTCCAGCGGGAGGGTGGTTGGCAGAGTCAAAGTAGGGAAGA  
ATGAAGAGTCTAAACCTACAAAAATAATGAGTGGAAATCCAGACCGTCTCAAAAA  
ACAGAGCAGACCTGACCGAGATGGTCAAGAAGATAACAGCATGAACAGGGG  
AGACTTCAAGCAGATTACTTTGGCAACAGGGGCAGGCAAAACCAAGAACTCC  
CAAAAGCAGTTATAGAGGAGATAGGAAGACACAAGAGAGATTAGTTCTTATA  
CCATTAAGGGCAGCGGCAGAGTCACTGCTACCAAGTATATGAGATTGAAACACCC  
AAGCATCTCTTTTAACTAAGGATAGGGGACATGAAAGAGGGGGACATGGCAA  
CCGGGATAACCTATGCATCATACGGGTACTTCTGCCAAATGCCTCAACCAAAGC  
TCAGAGCTGCTATGGTAGAATACTCATACATATTCTTAGATGAATACCATTTGTGC  
CACTCCTGAACAACCTGGCAATTATCGGGAAGATCCACAGATTTTTCAGAGAGTAT  
AAGGGTTGTGCGCCATGACTGCCACGCCAGCGGTCGGTGACCACAACAGGT

FIGURE 24-2

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CAAAAGCACCCAATAGAGGAATTCATAGCCCCCGAGGTAATGAAAGGGGAGG  
ATCTTGGTAGTCAGTTCCTTGATATAGCAGGGTTAAAAATACCAAGTGGATGAGA  
TGAAAGGCAATATGTTGGTTTTTGTACCAACGAGAAACATGGCAGTAGAGGTA  
GCAAAGAAGCTAAAAGCTAAGGGCTATAACTCTGGATACTATTACAGTGGAGA  
GGATCCAGCCAATCTGAGAGTTGTGACATCACAAATCCCCCTATGTAATCGTGGC  
TACAAATGCTATTGAATCAGGAGTGACACTACCAGATTTGGACACGGTTATAGA  
CACGGGGTTGAAATGTGAAAAGAGGGTGAGGGTATCATCAAAGATACCCTTCA  
TCGTAACAGGCCTTAAGAGGATGGCCGTGACTGTGGGTGAGCAGGCGCAGCG  
TAGGGGCAGAGTAGGTAGAGTGAAACCCGGGAGGTATTATAGGAGCCAGGAA  
ACAGCAACAGGGTCAAAGGACTACCACTATGACCTCTTGCAGGCACAAAGATA  
CGGGATTGAGGATGGAATCAACGTGACGAAATCCTTTAGGGAGATGAATTACG  
ATTGGAGCCTATACGAGGAGGACAGCCTACTAATAACCCAGCTGGAAATACTA  
AATAATCTACTCATCTCAGAAGACTTGCCAGCCGCTGTTAAGAACATAATGGCC  
AGGACTGATCACCAGAGCCAATCCAACCTTGCATACAACAGCTATGAAGTCCA  
GGTCCCGGTCTGTTCCCAAAAATAAGGAATGGAGAAGTCACAGACACCTACG  
AAAATTACTCGTTTCTAAATGCCAGAAAGTTAGGGGAGGATGTGCCCGTGATA  
TCTACGCTACTGAAGATGAGGATCTGGCAGTTGACCTCTTAGGGCTAGACTGG  
CCTGATCCTGGGAACCAAGCAGGAGTAGTGGAGACTGGTAAAGCACTGAAGCAAGT  
GACCGGGTTGTCTCGGCTGAAAATGCCCTACTAGTGGCTTTATTTGGGTATGT  
GGGTACCAGGCTCTCTCAAAGAGGCATGTCCCAATGATAACAGACATATATAC  
CATCGAGGACCAGAGACTAGAAGACACCACCCACCTCCAGTATGCACCCAACG  
CCATAAAAACCGATGGGACAGAGACTGAACTGAAAGAAGTGGCGTCGGGTGA  
CGTGGAATAAATCATGGGAGCCATTTGAGATTATGCAGCTGGGGGACTGGAGT  
TTGTTAAATCCCAAGCAGAAAAGATAAAAACAGCTCCTTTGTTTAAAGAAAACG  
CAGAAGCCGCAAAAGGGTATGTCCAAAATTCATTGACTCATTAAATTGAAAATA  
AAGAAGAAATAATCAGATATGGTTTGTGGGGAACACACACAGCACTATACAAA  
AGCATAGCTGCAAGACTGGGGCATGAAACAGCGTTTGGCCACACTAGTGTAA  
GTGGCTAGCTTTTGGAGGGGAATCAGTGTGAGACCACGTCAAGCAGGCGGCA  
GTTGATTTAGTGGTCTATTATGTGATGAATAAGCCTTCCCTCCAGGTGACTCC  
GAGACACAGCAAGAAGGGAGGCGATTTCGTGCAAGCCTGTTTCATCTCCGCACT  
GGCAACCTACACATACAAAACCTTGAATTACCACAATCTCTCTAAAGTGGTGA  
ACCAGCCCTGGCTTACCTCCCCTATGCTACCAGCGCATTAAAAATGTTACCCC  
AACGCGGCTGGAGAGCGTGGTGATACTGAGCACCACGATATATAAAACATACC  
TCTCTATAAGGAAGGGGAAGAGTGATGGATTGCTGGGTACGGGGATAAGTGC  
AGCCATGGAAATCCTGTCAAAAACCCAGTATCGGTAGGTATATCTGTGATGTT  
GGGGTAGGGGCAATCGCTGCGCACAAACGCTATTGAGTCCAGTGAACAGAAA  
AGGACCCTACTTATGAAGGTGTTTGTAAAGAACTTCTTGGATCAGGCTGCAACA  
GATGAGCTGGTAAAAGAAAACCCAGAAAAAATTATAATGGCCTTATTTGAAGCA  
GTCCAGACAATTGGTAACCCCTGAGACTAATATAACCACTGTATGGGGTTTAC  
TACAAAGGTTGGGAGGCCAAGGAACTATCTGAGAGGACAGCAGGCAGAACT  
TATTCACATTGATAATGTTTGAAGCCTTCGAGTTATTAGGGATGGACTCACAAG  
GGAAAAATAAGGAACCTGTCCGGAATTACATTTTGGATTGATATACGGCCTAC  
ACAAGCAAATCAACAGAGGGCTGAAGAAAATGGTACTGGGGTGGGCCCTGC  
ACCTTTAGTTGTGACTGGACCCCTAGTGACGAGAGGATCAGATTGCCAACAG  
ACAATATTTGAGGGTAGAAACCAAGGTGCCCATGTGGCTATGAGATGAAAGCT  
TTCAAAAATGTAGGTGGCAAACTTACCAAAGTGGAGGAGAGCGGGCCTTTCT  
ATGTAGAAACAGACCTGGTAGGGGACCAAGTCAACTACAGAGTCACCAAGTATT  
ACGATGACAACCTCAGAGAGATAAAACCAAGTAGCAAAGTTGGAAGGACAGGTA  
GAGCACTACTACAAAGGGGTACAGCAAAAATTGACTACAGTAAAGGAAAAAT  
GCTCTTGGCCACTGACAAGTGGGAGGTGGAACATGGTGTCTATAACCAGGTTAG  
CTAAGAGATATACTGGGGTTCGGGTTCAATGGTGCATACTTAGGTGACGAGCCC  
AATCACCGTGTCTAGTGGAGAGGGACTGTGCAACTATAACCAAAAACAGT  
ACAGTTTCTAAAAATGAAGAAGGGGTGTGCGTTACCTATGACCTGACCATCTC  
CAATCTGACCAGGCTCATCGAACTAGTACACAGGAACAATCTTGAAGAGAAGG

FIGURE 24-3

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AAATACCCACCGCTACGGTCACCACATGGCTAGCTTACACCTTCGTGAATGAAG  
ACGTAGGGACTATAAAACCAGTACTAGGAGAGAGAGTAATCCCCGACCCTGTA  
GTTGATATCAATTTACAACCAGAGGTGCAAGTGGACACGTCAGAGGTTGGGAT  
CACAATAATTGGAAGGGAAACCTGATGACAACGGGAGTGACACCTGTCTTGG  
AAAAAGTAGAGCCTGACGCCAGCGACAACCAAACTCGGTGAAGATCGGGTTG  
GATGAGGGTAATTACCCAGGGCCTGGAATACAGACACATACTAACAGAAGA  
AATACACAACAGGGATGCGAGGCCCTTCATCATGATCCTGGGCTCAAGGAATT  
CCATATCAAATAGGGCAAAGACTGCTAGAAATATAAATCTGTACACAGGAAATG  
ACCCAGGGAAATACGAGACTTGATGGCTGCAGGGCGCATGTTAGTAGTACGA  
CTGAGGGATGTCGACCCTGAGCTGTCTGAAATGGTCGATTTCAAGGGGACTTT  
TTTAGATAGGGAGGCCCTGGAGGCTCTAAGTCTCGGGCAACCTAAACCGAAGC  
AGGTTACCAAGGAAGCTGTTAGGAATTTGATAGAACAGAAAAAGATGTGGAG  
ATCCCTAACTGGTTTGCATCAGATGACCCAGTATTTCTGGAAGTGGCCTTAAAA  
AATGATAAGTACTACTTAGTAGGAGATGTTGGAGAGGTAAAAGATCAAGCTAA  
AGCACTTGGGGCCAGGATCAGACAAGAATTATAAAGGAGGTAGGCTCAAGG  
ACGTATGCCATGAAGCTATCTAGCTGGTTCTCAAGGCATCAAACAAACAGATG  
AGTTTAACTCCACTGTTTGAGGAATTGTTGCTACGGTGCCACCTGCAACTAAG  
AGCAATAAGGGGCACATGGCATCAGCTTACCAATTGGCACAGGGTAAGTGGGA  
GCCCCCTCGGTTGCGGGGTGCACCTAGGTACAATACCAGCCAGAAGGGTGAAG  
ATACACCCATATGAAGCTTACCTGAAGTTGAAAGATTTTCATAGAAGAAGAAGAG  
AAGAAACCTAGGGTTAAGGATACAGTAATAAGAGAGCACAACAAATGGATGAT  
TAAAAAAATAAGGTTTCAAGGAAACCTCAACACCAAGAAATGCTCAACCTGG  
GAAACTATCTGAACAGTTGGACAGGGAGGGGCGCAAGAGGAACATCTACAAC  
CACCAGATTGGTACTATAATGTCAAGTGCAGGCATAAGGCTGGAGAAATTGCC  
AATAGTGAGGGCCCAACCGACACCAAAACCTTTTCATGAGGCAATAAGAGATA  
AGATAGACAAGAGTGAAAACCGGCAAAATCCAGAATTGCACAACAAATTGTTG  
GAGATTTTCCACACGATAGCCCCAACCCACCCTGAAACACACCTACGGTGAGGT  
GACGTGGGAGCAACTTGAGGCGGGGATAAATAGAAAGGGGCGCAGGCTTC  
CTGGAGAAGAAGAACATCGGAGAAGTATTGGATTTCAGAAAAGCACCTGGTAGA  
ACAATTGGTCAGGGATCTGAAGGCCGGGAGAAAGATAAAATATTATGAAACTG  
CAATACCAAAAAATGAGAAGAGAGATGTCAGTGATGACTGGCAGGCAGGGGA  
CCTGGTGGTTGAGAAGAGGCCAAGAGTTATCCAATACCCTGAAGCCAAGACAA  
GGCTAGCCATCACTAAGGTCATGTATAACTGGGTGAAACAGCAGCCCCGTTGTG  
ATTCCAGGATATGAAGGAAAGACCCCTTGTTCAACATCTTTGATAAAGTGAGA  
AAGGAATGGGACTCGTTCAATGAGCCAGTGCCGTAAGTTTTGACACCAAGC  
CTGGGACACTCAAGTGACTAGTAAGGATCTGCAACTTATTGGAGAAATCCAGA  
AATATTACTATAAGAAGGAGTGGCACAAGTTCATTGACACCATCACCGACCACA  
TGACAGAAGTACCAGTTATAACAGCAGATGGTGAAGTATATATAAGAAATGGG  
CAGAGAGGGAGCGGCCAGCCAGACACAAGTGCTGGCAACAGCATGTTAAATG  
TCCTGACAATGATGTACGCCCTTCTGCGAAAGCACAGGGGTACCGTACAAGAGT  
TTCAACAGGGTGGCAAGGATCCACGTCTGTGGGGATGATGGCTTCTTAATAAC  
TGAAAAAGGGTTAGGGCTGAAATTTGCTAACAAAGGGATGCAGATTCTTCATG  
AAGCAGGCAAACCTCAGAAGATAACGGAAGGGGAAAAGATGAAAGTTGCCTAT  
AGATTTGAGGATATAGAGTTCTGTTCTCATACCCAGTCCCTGTTAGGTGGTCC  
GACAACACCAGTAGTCACATGGCCGGGAGAGACACCGCTGTGATACTATCAAA  
GATGGCAACAAGATTGGATTCAAGTGGAGAGAGGGGTACCACAGCATATGAAA  
AAGCGGTAGCCTTCAGTTTCTTGCTGATGTATTCTGGAACCCGCTTGTAGGA  
GGATTTGCCTGTTGGTCCCTTTCGCAACAGCCAGAGACAGACCCATCAAACATG  
CCACTTATTATTACAAAGGTGATCCAATAGGGGCCTATAAAGATGTAATAGGTC  
GGAATCTAAGTGAAGTGAAGAGAACAGGCTTTGAGAAATTGGCAAATCTAAC  
CTAAGCCTGTCCACGTTGGGGATCTGGACTAAGCACACAAGCAAAAGAATAAT  
TCAGGACTGTGTTGCCATTGGGAAAGAAGAGGGCAACTGGCTAGTTAACGCCG  
ACAGGCTGATATCCAGCAAAACTGGCCACTTATACATACCTGATAAAGGCTTTA  
CATTACAAGGAAAGCATTATGAGCAACTGCAGCTAAGAACAGAGACAAACCCG

FIGURE 24-4

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GTCATGGGGGTTGGGACTGAGAGATACAAGTTAGGTCCCATAGTCAATCTGCT  
GCTGAGAAGGTTGAAAATTCTGCTCATGACGGCCGTCGGCGTCAGCAGCTGAg  
acaaaatgtatatattgtaataaattaatccatgtacAATTCCGCCCCCTCTCCCTCCCCCCCCCTAACG  
TTACTGGCCGAAGCCGCTTGAATAAGGCCGGTGTGCGTTTGTCTATATGTTAT  
TTTCCACCATATTGCCGTCTTTTGGCAATGTGAGGGCCCCGAAACCTGGCCCTG  
TCTTCTTGACGAGCATTCTAGGGGTCTTTCCCCTCTCGCCAAAGGAATGCAAG  
GTCTGTTGAATGTCGTGAAGGAAGCAGTTCCTCTGGAAGCTTCTTGAAGACAAA  
CAACGTCTGTAGCGACCCTTTGCAGGCAGCGGAACCCCCACCTGGCGACAGG  
TGCCCTCTGCGGCCAAAAGCCACGTGTATAAGATACACCTGCAAAGGCGGCACA  
ACCCACAGTGCCACGTTGTGAGTTGGATAGTTGTGGAAAGAGTCAAATGGCTCT  
CCTCAAGCGTATTCAACAAGGGGCTGAAGGATGCCCAGAAGGTACCCATTGT  
ATGGGATCTGATCTGGGGCCTCGGTGCACATGCTTTACATGTGTTTGTGCGAG  
GTTAAAAAACGTCTAGGCCCCCGAACCACGGGGACGTGGTTTTCTTTGAAA  
AACACGATGATAAGCTTGCCACAACcatgaccgagtacaagcccacggtgcgcctcgccacccgcgacga  
cgccccgggcccgtacgcacccctcgccgcccgcgttcgcccactaccccgccacgcgcccacccgtcgaccggaccgcccacatc  
gagcgggtcaccgagctgcaagaactctctctacgcgcgtcgggtcgcacatcggaaggtgtgggtcgcggacgacggcgccc  
gcggtggcggtctgaccacgcccggagagcgtcgaagcggggcggtgttcgccgagatcgccccgcgcatggccgagttgag  
cggtccccggctggccgcgagcaacagatggaaggcctcctggcgccgcacggcccaaggagcccgcgtggttcctggccac  
cgtcggcgtctcgccgaccaccagggaagggtctgggcagcgcgcgtcgtctccccgagtgaggcgggcgagcgcgcgcg  
gggtgcccgccttcttgagacctccgcgccccgcaacctccccctctacgagcggctcgggttcaccgtcaccgcccgcgacgtc  
gcccgaaggaccgcgcgacctggtgcatgaccgcaagcccgggtgccTGAaccccggcccacgaccgacgcgcccgaccg  
aaaggagcgcacgaccccatgaaATGCATCGATCGTACGAATTAAACGCCGACAGGCTGATAT  
CCAGCAAACTGGCCACTTATACATACCTGATAAAGGCTTTACATTACAAGGAA  
AGCATTATGAGCAACTGCAGCTAAGAACAGAGACAAACCCGGTCATGGGGGTT  
GGGACTGAGAGATACAAGTTAGGTCCCATAGTCAATCTGCTGCTGAGAAGGTT  
GAAAATTCTGCTCATGACGGCCGTCGGCGTCAGCAGCTGAacaaaatgtatatattgtaata  
aattaatccatgtacatagtgatataaatatagttgggaccgtccacctcaagaagacgacagcccaacacgcacagctaaacagtag  
tcaagattatctacctaagataaacactacattaatgcacacagcacttttagctgtatgaggatacgcccgacgtctatagttggactagg  
gaagaccttaacagcccc

FIGURE 24-5





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Gtatacgagaattagaaaaggcactcgtatagctattgggcaattaaaaataataattaggcctaggtacatggcacgtgccagccccct  
gatggggcgacactccaccatgaatcactcccctgtagggaactactgtcttcacgcagaaagcgctagccatggcgtagtatgag  
tgtcgtgcagcctccaggacccccctcccggagagccatagtggctgcggaaccggtagtacaccggaatgccaggacgac  
cgggtcctttcttgataaaccgctcaatgcctggagattgggctgccccgcaagactgtagccgagtagtgggtcgcgaa  
aggccttggtgactgcctgatagggtgctgcgagtgccccgggaggtctcgtagaccgtgcaccATGAGCACGAATC  
CTAAACCTCAAAGAAAAACCAAACGTAACACCAACCGTCGCCACAGGACGTC  
AAGTTCCCGGGTGGCGGTACAGATCGTTGGTGGAGTTTACTTGTGCGCGCAG  
GGGCCCTAGATTGGGTGTGCGCGCGACGAGGAAGACTTCCGAGCGGTGCGAA  
CCTCGAGGTAGACGTCAGCCTATCCCCAAGGCACGTCGGCCCCGAGGGCAGGA  
CCTGGGCTCAGCCCGGGTACCCTTGGCCCCCTCTATGGCAATGAGGGTTGCGGG  
TGGGCGGGATGGCTCCTGTCTCCCCGTGGCTCTCGGCCTAGCTGGGGCCCCAC  
AGACCCCCGGCGTAGGTGCGCGCAATTTGGGTAAAGGTACATCGATACCCCTTACGT  
GCGGCTTCGCCGACCTCATGGGGTACATACCGCTCGTCGGCGCCCCCTCTTGA  
GGCGCTGCCAGGGCCCTGGCGCATGGCGTCCGGGTTCTGGAAGACGGCGTGA  
ACTATGCAACAGGGAACCTTCTGTTGCTCTTTCTCTATCTTCTTCTGGCCCT  
GCTCTCTTGCTGACCGTGCCCGCTTCAGCCTACCAAGTGCGCAATTCCTCGGG  
GCTTTACCATGTACCAATGATTGCCCTAACTCGAGTATTGTGTACGAGGCGGC  
CGATGCCATCCTGCACACTCCGGGGTGTGTCCCTTGCCTTCGGAGGGTAACG  
CCTCGAGGTGTTGGGTGGCGGTGACCCCCACGGTGGCCACCAGGGACGGCAA  
ACTCCCCACAACGCAGCTTCGACGTCATATCGATCTGCTTGTCCGGAGCGCCA  
CCCTCTGCTCGGCCCTCTACGTGGGGGACCTGTGCGGGTCTGTCTTCTTGTG  
GTCAACTGTTTACCTTCTCTCCAGGCGCCACTGGACGACGCAAGACTGCAATT  
GTTCTATCTATCCCGGCCATATAACGGGTACATCGCATGGCATGGGATATGATGA  
TGAAGTGGTCCCCCTACGGCAGCGTTGGTGGTAGCTCAGCTGCTCCGGATCCCA  
CAAGCCATCATGGACATGATCGCTGGTGTCACTGGGGAGTCTGGCGGGCAT  
AGCGTATTTCTCCATGGTGGGGAAGTGGGCGAAGGTCCTGGTAGTGCTGTGC  
TATTTGCCGGCGTCGACGCGGAAACCCACGTCACCGGGGGAAGTGCCGGCCG  
CACCACGGCTGGGCTTGTGTTGCTCTCTTACACCAGGCGCCAAGCAGAACATCC  
AACTGATCAACACCAACGGCAGTTGGCACATCAATAGCACGGCCTTGAACCTG  
AATGAAAGCCTTAACACCGGCTGGTTAGCAGGGCTCTTCTATCAGCACAAATTC  
AACTCTCAGGCTGTCTGAGAGGTTGGCCAGCTGCCGACGCCTTACCGATTTT  
GCCAGGGCTGGGGTCCCTATCAGTTATGCCAACGGAAGCGGCCTCGACGAAC  
GCCCTACTGCTGGCACTACCCTCCAAGACCTTGTGGCATTGTGCCCGCAAAG  
AGCGTGTGTGGCCCGGTATATTGCTTCACTCCAGCCCCGTGGTGGTGGGAAC  
GACCGACAGGTGCGGCGCGCCTACCTACAGCTGGGGTGCAATGATACGGAT  
GTCTTCGTCTTAACAACACCAAGGCCACCGCTGGGCAATTGGTTCGGTTGTACC  
TGGATGAACTCAACTGGATTACCAAAAGTGTGCGGAGCGCCCCCTTGTGTCT  
CGGAGGGGTGGGCAACAACACCTTGTCTGCCCCACTGATTGTTTCCGCAAGC  
ATCCGGAAGCCACATACTCTCGGTGCGGCTCCGGTCCCTGGATTACACCCAGG  
TGCATGGTCTGACTACCCGTATAGGCTTTGGCACTATCCTTGTACCATCAATTAC  
ACCATATTCAAAGTCAGGATGTACGTGGGAGGGGTCGAGCACAGGCTGGAAG  
CGGCCTGCAACTGGACGCGGGGCGAACCGTGTGATCTGGAAGACAGGGACAG  
GTCCGAGCTCAGCCCATTGTGCTGTCCACCACACAGTGGCAGGTCTTCCGT  
GTTCTTTACGACCCTGCCAGCCTTGTCCACCGGCCTCATCCACCTCCACCAGA  
ACATTGTGGACGTGCAGTACTTGTACGGGGTAGGGTCAAGCATCGCGTCTCTGG  
GCCATTAAGTGGGAGTACGTGCTTCTCTGTTCTCTGCTTGCAGACGCGCGC  
GTCTGCTCCTGCTTGTGGATGATGTTACTCATATCCCAAGCGGAGGCGGCTTGT  
GAGAACCTCGTAATACTCAATGCAGCATCCCTGGCCGGGACGCACGGTCTTGT  
GTCTTCTCTCGTGTCTTCTGCTTGTGCGTGGTATCTGAAGGGTAGGTGGGTGCC

FIGURE 26-1

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CGGAGCGGTCTACGCCTTCTACGGGATGTGGCCTCTCCTCCTGCTCCTGCTGG  
CGTTGCCTCAGCGGGCATACGCACTGGACACGGAGGTGGCCGCGTCTGCTGG  
CGGCGTTGTTCTTGTTCGGTTAATGGCGCTGACTCTGTTCGCCATATTACAAGCG  
CTACATCAGCTGGTGCATGTGGTGGCTTCAGTATTTTCTGACCAGAGTAGAAGC  
GCAACTGCACGTGTGGGTTCCTCCCTCAACGTCCGGGGGGGGCGCGATGCC  
GTCATCTTACTCATGTGTGTGTACACCCGACTCTGGTATTTGACATCACCAAAC  
TACTCCTGGCCATCTTCGGACCCCTTTGGATTCTTCAAGCCAGTTTGCTTAAAGT  
CCCCTACTTCGTGCGCGTTCAAGGCCTTCTCCGGATCTGCGCGCTAGCGCGGA  
AGATAGCCGAGGTCAATTACGTGCAAAATGGCCATCATCAAGTTAGGGGCGCTT  
ACTGGCACCTATGTGTATAACCATCTACCCCTCTTCGAGACTGGGCGCACAAAC  
GGCCTGCGAGATCTGGCCGTGGCTGTGGAACCAAGTCGTCTTCTCCCGAATGGA  
GACCAAGCTCATCACGTGGGGGGCAGATACCGCCGCGTGGGCTGACATCATC  
AACGGCTTGCCCGTCTCTGCCCGTAGGGGCCAGGAGATACTGCTTGGGCCAGC  
CGACGGAATGGTCTCCAAGGGGTGGAGGTTGCTGGCGCCCATCACGGCGTAC  
GCCACGAGACGAGAGGCCTCCTAGGGTGTATAATCACCAGCCTGACTGGCCG  
GGACAAAAACCAAGTGGAGGGTGAGGTCCAGATCGTGTCAACTGTACCCAAA  
CCTTCTGGCAACGTGCATCAATGGGGTATGCTGGACTGTCTACCACGGGGCC  
GGAACGAGGACCATCGCATCACCAAGGGTCTGTATCCAGATGTATACCAA  
TGTGGACCAAGACCTTGTGGGCTGGCCCGCTCCTCAAGGTTCCCGCTCATTGA  
CACCTGCACTGCGGCTCCTCGGACCTTTACCTGGTCACGAGGCACGCCGAT  
GTCATTCCCGTGCGCCGGCGAGGTGATAGCAGGGGTAGCCTGCTTTCGCCCCG  
GCCCATTCTTCTTGAAGGCTCCTCGGGGGGTCCGCTGTTGTGCCCGCGG  
GACACGCCGTGGGCCTATTACAGGGCCGCGGTGTGCACCCGTGGAGTGGCTAA  
GGCGGTGGACTTTATCCCTGTGGAGAACCCTAGAGACAACCATGAGATCCCCGG  
TGTTACGGACAACCTCCTCTCCACCAGCAGTGCCCCAGAGCTTCCAGGTGGCC  
CACCTGCATGCTCCCAACGGCAGCGGTAAGAGCACCAAGGTCCCGGCTGCGTA  
CGCAGCCCAGGGCTACAAGGTGTTGGTGTCTCAACCCCTCTGTTGCTGCAACGC  
TGGGCTTTGGTGCTTACATGTCCAAGGCCCATGGGGTTGATCCTAATATCAGGA  
CCGGGGTGAGAACAATTACCACTGGCAGCCCCATCACGTACTCCACCTACGGC  
AAGTTCCTTGCCGACGGCGGGTGTCTCAGGAGGTGCTTATGACATAATAATTTGT  
GACGAGTGCCACTCCACGGATGCCACATCCATCTTGGGCATCGGCACTGTCT  
TGACCAAGCAGAGACTGCGGGGGCGAGACTGGTGTGTGCTCGCCACTGCTACC  
CCTCCGGGCTCCGTCACTGTGTCCCATCCTAACATCGAGGAGGTTGCTCTGTCC  
ACCACCGGAGAGATCCCCCTTTACGGCAAGGCTATCCCCCTCGAGGTGATCAA  
GGGGGAAGACATCTCATCTTCTGCCACTCAAAGAAGAAGTGCGCAGGACTCG  
CCGCGAAGCTGGTTCGATTGGGCATCAATGCCGTGGCCTACTACCGCGTCTT  
GACGTGTCTGTATCCCGACCAAGCGGCGATGTTGTCTGCTGTCGACCGATGC  
TCTCATGACTGGCTTTACCGGCGACTTCGACTCTGTGATAGACTGCAACACGTG  
TGTACTCAGACAGTCGATTTACGCCTTGACCCTACCTTTACCATTGAGACAAC  
CACGCTCCCCCAGGATGCTGTCTCCAGGACTCAACGCCGGGGCAGGACTGGC  
AGGGGGAAGCCAGGCATCTACAGATTTGTGGCACCAGGGGGAGCGCCCCCTCG  
GCATGTTTCGACTCGTCCGTCTCTGTGAGTGCTATGACGCGGGCTGTGCTTGG  
TATGAGCTCACGCCCCGCGAGACTACAGTTAGGCTACGAGCGTACATGAACAC  
CCCGGGGCTTCCCGTGTGCCAGGACCATCTTGAATTTTGGGAGGGCGTCTTTA  
CGGGCCTCACTCATATAGATGCCCACTTTCTATCCCAGACAAAGCAGAGTGGG  
GAGAACTTTCTTACCTGGTAGCGTACCAAGCCACCGTGTGCGCTAGGGTCA  
AGCCCCCTCCCCATCGTGGGACCAAGATGTGGAAGTGTGATCCGCTTAAAC  
CCACCTCCATGGGCCAACACCCCTGCTATACAGACTGGGCGCTGTTTCAAGT  
GAAGTCACCCTGACGCACCCAATCACCAAATACATCATGACATGCATGTCGGCC  
GACCTGGAGGTGCTCACGAGCACCTGGGTGCTCGTTGGCGGCGTCTTGGCTG  
CTCTGGCCGCGTATTGCTGTCAACAGGCTGCGTGGTTCATAGTGGGCAGGATT  
GTCTTGTCCGGGAAGCCGGCAATTATACCTGACAGGGAGGTTCTCTACCAGGA  
GTTTCGATGAGATGGAAGAGTGCTCTCAGCACTTACCGTACATCGAGCAAGGGA  
TGATGCTCGCTGAGCAGTTCAAGCAGAAGGCCCTCGGCCTCCTGCAGACCGCG

FIGURE 26-2

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TCCTCGCAAGCAGAGGTTATCACCCCTGCTGTCCAGACCAACTGGCAGAACT  
CGAGGTCTTCTGGGCGAAGCACATGTGGAATTTTCATCAGTGGGATACAATACTT  
GGCGGGCCTGTCAACGCTGCCTGGTAACCCCGCCATTGCTTCATTGATGGCTTT  
TACAGCTGCCGTCAACAGCCCACTAACCCTGGCCAAACCCCTCCTCTTCAACAT  
ATTGGGGGGGTGGGTGGCTGCCAGCTCGCCGCCCCCGGTGCCGCTACCGCC  
TTTGTGGGCGCTGGCTTAGCTGGCGCCGCCATCGGCAGCGTTGGACTGGGA  
AGGTCCTCGTGGACATTCTTGCAAGGTATGGCGCGGGCGTGGCGGGAGCTCT  
TGAGCCTTCAAGATCATGAGCGGTGAGGTCCCCTCCACGGAGGACCTGGTCA  
ATCTGCTGCCCCGCCATCCTCTCGCTGGAGCCCTTGTAGTCGGTGTGGTCTGC  
GCAGCAATACTGCGCCGGCAGCTTGGCCCCGGCGAGGGGGCAGTGCAATGGA  
TGAACCGGCTAATAGCCTTCGCCTCCCGGGGAACCATGTTTCCCCACGCAC  
TACGTGCCGGAGAGCGATGCAGCGCCCGCGTCACTGCCATACTCAGCAGCCT  
CACTGTAACCCAGCTCCTGATcgCTAGaccatggggtaccgagCGTTACTGGCCGAAGCC  
GCTTGGAAATAAGGCCGGTGTGCGTTTGTCTATATGTTATTTCCACCATATTGCC  
GTCTTTTGGCAATGTGAGGGCCCCGGAACCTGGCCCTGTCTTCTTGACGAGCA  
TTCCTAGGGGTCTTCCCCCTCTCGCCAAAGGAATGCAAGGTCTGTTGAATGTG  
TGAAGGAAGCAGTTCCTCTGGAAGCTTCTTGAAGACAAACAACGTCTGTAGCG  
ACCCCTTTCAGGCAGCGGAACCCCCCACTGGCGACAGGTGCCTCTGCGGCCA  
AAAGCCACGTGTATAAGATACACCTGCAAAGGCGGCACAACCCCACTGCCACG  
TTGTGAGTTGGATAGTTGTGGAAGAGTCAAATGGCTCTCCTCAAGCGTATTCA  
ACAAGGGGCTGAAGGATGCCCAGAAGGTACCCCACTGTATGGGATCTGATCTG  
GGGCTCGGTGCACATGCTTTACATGTGTTTAGTCGAGGTAAAAAACGTCTAG  
GCCCCCGAACCACGGGGACGTGTTTCTTTGAAAAACACGATGATAATAT  
GGAGTTGATCACAATGAACCTTTATACAAAACATACAAAACAAAAACCGTCGG  
GGTGAGGAACCTGTTTATGATCAGGCAGGTGATCCCTTATTTGGTGAAAGGG  
GAGCAGTCCACCCTCAATCGACGCTAAAGCTCCCAACAAGAGAGGGGAACGC  
GATGTTCCAACCAACTTGGCATCCTTACCAAAAAGAGGTGACTGCAGGTGCGG  
TAATAGCAGAGGACCTGTGAGCGGGATCTACCTGAAGCCAGGGCCACTATTTT  
ACCGGACTATAAAGGTCCCGTCTATCAGGGCCCCGCTGGAGCTCTTTGAG  
GAGGATCCATGTGTGAAACGACTAAACGGATAGGGAGAGTAAGTGAAGT  
ACGGAAGCTGTACCACTTTATGTGTGTATAGATGGATGTATAATAAAAA  
GTGCCACGAGAAGTTACCAAGGGGTGTTCAAGGTGGGTCCATAATAGGCTTGAC  
TGCCCTCTATGGGTCAAAAGTTGCTCAGACACGAAAGAAGAGGAGCAACAag  
cttGCATTGTTGGCGTGGGCAATAATAGCTATAGTTTTGTTTCAAGTTACAATGG  
AGAAAAACATAACACAGTGGAAACctgagTGGTTTGACCTGGAGGTGACTGACCAT  
CACCGGGATTACTTCGCTGAGTCCATATTAGTGGTGGTAGTAGCCCTCTTGGGT  
GGCAGATATGTACTTTGGTTACTGGTTACATACATGGTCTTATCAGAACAGAAG  
GCCTTAGGGATTCAATGATGATCAGGGGAAGTGGTGATGATGGGCAACTTGCT  
AACCATAACAATATTGAAGTGGTGACATACTTCTTGCTGCTGTACCTACTGCT  
GAGGGAGGAGAGCGTAAAGAAGTGGGTCTTACTCTTATACCACATCTTAGTGG  
TACACCAATCAAATCTGTAATTGTGATCCTACTGATGATTGGGGATGTGGTAA  
AGGCCGATTACGGGGGCAAGAGTACTTGGGGAAAATAGACCTCTGTTTACA  
ACAGTAGTACTAATCGTCATAGGTTTAAATCATAGCTAGGCGTGACCCAACATA  
GTGCCACTGGTAACAATAATGGCAGCACTGAGGGTCACTGAAGTACCCACCA  
GCCTGGAGTTGACATCGCTGTGGCGGTCACTGACTATAACCCTACTGATGGTTA  
GCTATGTGACAGATTATTTAGATATAAAAAATGGTTACAGTGCACTTCTCAGCCT  
GGTATCTGGGGTGTCTTGATAAGAAGCCTAATATACCTAGGTAGAATCGAGAT  
GCCAGAGGTAACATCCCAAACCTGGAGACCACTAATTTAATACTATTATTTG  
ATCTCAACAACAATTGTAACGAGGTGGAAGGTGACGTGGCTGGCCTATTGTT  
GCAATGTGTGCCTATCTTATTGCTGGTCACAACCTTGTGGGCCGACTTCTTAAC  
CCTAATACTGATCCTGCCTACCTATGAATTGGTTAAATTATACTATCTGAAAAC  
GTTAGGACTGATATAGAAAGAAGTTGGCTAGGGGGGATAGACTATACAAGAGT  
TGACTCCATCTACGACGTTGATGAGAGTGGAGAGGGCGTATATCTTTTCCATC  
AAGGCAGAAAGCACAGGGGAATTTTCTATACTCTTGCCCTTATCAAAGCAAC

FIGURE 26-3

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ACTGATAAGTTGCGTCAGCAGTAAATGGCAGCTAATATACATGAGTTACTTAAAC  
TTTGGACTTTATGTACTACATGCACAGGAAAGTTATAGAAGAGATCTCAGGAGG  
TACCAACATAATATCCAGGTTAGTGGCAGCACTCATAGAGCTGAACCTGGTCCAT  
GGAAGAAGAGGAGAGCAAAGGCTTAAAGAAGTTTTATCTATTGTCTGGAAGGT  
TGAGAAACCTAATAATAAAACATAAGGTAAGGAATGAGACCGTGGCTTCTTGGT  
ACGGGGAGGAGGAAGTCTACGGTATGCCAAAGATCATGACTATAATCAAGGCC  
AGTACACTGAGTAAGAGCAGGCACTGCATAATATGCACTGTATGTGAGGGCCG  
AGAGTGGAAAGGTGGCACCTGCCCAAATGTGGACGCCATGGGAAGCCGATA  
ACGTGTGGGATGTCGCTAGCAGATTTTGAAGAAAGACACTATAAAAGAATCTTT  
ATAAGGGAAGGCAACTTTGAGGGTATGTGCAGCCGATGCCAGGGAAAGCATA  
GGAGGTTTGAAATGGACCGGGAACCTAAGAGTGCCAGATACTGTGCTGAGTGT  
AATAGGCTGCATCCTGCTGAGGAAGGTGACTTTTGGGCAGAGTCGAGCATGTT  
GGGCCTCAAAATCACCTACTTTGCGCTGATGGATGGAAAGGTGTATGATATCAC  
AGAGTGGGCTGGATGCCAGCGTGTGGGAATCTCCCCAGATACCCACAGAGTCC  
CTTGTACATCTCATTGGTTCACGGATGCCTTTGAGGCAGGAATAACAATGGCT  
TTGTACAATATACCGCTAGGGGGCAACTATTTCTGAGAAACTTGCCCGTACTGG  
CAACTAAAGTAAAAATGCTCATGGTAGGCAACCTTGGAGAAGAAATTGGTAATC  
TGGAACATCTTGGGTGGATCCTAAGGGGGCCTGCCGTGTGTAAGAAGATCACA  
GAGCACGAAAAATGCCACATTAATATACTGGATAAACTAACCGCATTTTTCGGG  
ATCATGCCAAGGGGGACTACACCCAGAGCCCCGGTGAGGTTCCCTACGAGCTT  
ACTAAAAGTGAGGAGGGGTCTGGAGACTGGCTGGGCTTACACACACCAAGGC  
GGGATAAGTTGAGTCGACCATGTAACCGCGGAAAAGATCTACTGGTCTGTGA  
CAGCATGGGACGAACTAGAGTGGTTTGCCAAAGCAACAACAGGTTGACCGATG  
AGACAGAGTATGGCGTCAAGACTGACTCAGGGTGCCCAGACGGTGCCAGATG  
TTATGTGTTAAATCCAGAGGCCGTTAACATATCAGGATCCAAAGGGGCAGTCGT  
TCACCTCCAAAAGACAGGTGGAGAATTCACGTGTGTACCGCATCAGGCACAC  
CGGCTTTCTTCGACCTAAAAAACTTGAAAGGATGGTCAGGCTTGCCATATTTG  
AAGCCTCCAGCGGGAGGGTGGTTGGCAGAGTCAAAGTAGGGAAGAATGAAGA  
GTCTAAACCTACAAAAATAATGAGTGGAAATCCAGACCGTCTCAAAAAACACAGC  
AGACCTGACCGAGATGGTCAAGAAGATAAACCAGCATGAACAGGGGAGACTTCA  
AGCAGATTACTTTGGCAACAGGGGCAGGCAAAACCAACAGAACTCCCAAAAGCA  
GTTATAGAGGAGATAGGAAGACACAAGAGAGTATTAGTTCTTATACCATTAAGG  
GCAGCGGCAGAGTCAGTCTACCAGTATATGAGATTGAAACACCCAAGCATCTC  
TTTTAACCTAAGGATAGGGGACATGAAAGAGGGGGACATGGCAACCGGGATA  
ACCTATGCATCATACGGGTACTTCTGCCAAATGCCTCAACCAAGCTCAGAGCT  
GCTATGGTAGAATACTCATACATATTCTTAGATGAATACCATTTGCCCCTCCTG  
AACAACTGGCAATTATCGGGAAGATCCACAGATTTTCAGAGAGTATAAGGGTT  
GTCGCCATGACTGCCACGCCAGCAGGGTGGTGACCACAACAGGTCAAAAGC  
ACCCAATAGAGGAATTCATAGCCCCGAGGTAATGAAAGGGGAGGATCTTGGT  
AGTCAGTTCCCTTGATATAGCAGGGTTAAAAATACCAGTGGATGAGATGAAAGG  
CAATATGTTGGTTTTTTGTACCAACGAGAAACATGGCAGTAGAGGTAGCAAAGA  
AGCTAAAAGCTAAGGGCTATAACTCTGGATACTATTACAGTGGAGAGGATCCA  
GCCAATCTGAGAGTTGTGACATCACAATCCCCCTATGTAATCGTGGCTACAAAT  
GCTATTGAATCAGGAGTGACACTACCAGATTTGGACACGGTTATAGACACGGG  
GTTGAAATGTGAAAAGAGGGTGAGGGTATCATCAAAGATACCCTTCATCGTAA  
CAGGCCTTAAGAGGATGGCCGTGACTGTGGGTGAGCAGGCGCAGCGTAGGGG  
CAGAGTAGGTAGAGTGAAACCCGGGAGGTATTATAGGAGCCAGGAAACAGCA  
ACAGGGTCAAAGGACTACCAGTATGACCTCTTGCAAGGCACAAAGATAACGGGAT  
TGAGGATGGAATCAACGTGACGAAATCCTTTAGGGAGATGAATTACGATTGGA  
GCCTATACGAGGAGGACAGCCTACTAATAACCCAGCTGGAAATACTAAATAATC  
TACTCATCTCAGAAGACTTGCCAGCCGCTGTTAAGAACATAATGGCCAGGACTG  
ATCACCCAGAGCCAATCCAACCTTGATACAAACAGCTATGAAGTCCAGGTCCCG  
GTCCTGTTCCCAAAAATAAGGAATGGAGAAGTCACAGACACCTACGAAAATTAC  
TCGTTTCTAAATGCCAGAAAGTTAGGGGAGGATGTGCCCGTGATATCTACGCT

FIGURE 26-4

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ACTGAAGATGAGGATCTGGCAGTTGACCTCTTAGGGCTAGACTGGCCTGATCC  
TGGGAACCGAGCAGGTAGTGGAGACTGGTAAAGCACTGAAGCAAGTGACCGGG  
TTGTCCTCGGCTGAAAATGCCCTACTAGTGGCTTTATTTGGGTATGTGGGTAC  
CAGGCTCTCTCAAAGAGGCATGTCCCAATGATAACAGACATATATACCATCGAG  
GACCAGAGACTAGAAGACACCAACCCACCTCCAGTATGCACCCAACGCCATAAA  
AACCGATGGGACAGAGACTGAACTGAAAGAACTGGCGTCGGGTGACGTGGAA  
AAAATCATGGGAGCCATTTTCAGATTATGCAGCTGGGGGACTGGAGTTTGTTAA  
ATCCCAAGCAGAAAAGATAAAAAACAGCTCCTTTGTTTAAAGAAAACGCAGAAGC  
CGAAAAGGGTATGTCCAAAATTCATTGACTCATTAAATTGAAAATAAAGAAGA  
AATAATCAGATATGGTTTGTGGGGAACACACAGCACTATACAAAAGCATAGC  
TGCAAGACTGGGGCATGAAACAGCGTTTGCCACACTAGTTGTTAAAGTGGCTAG  
CTTTTGGAGGGGAATCAGTGTGATGAATAAGCCTTCCCTCCAGGTGACTCCGAGACACAG  
GTGGTCTATTATGTGATGAATAAGCCTTCCCTCCAGGTGACTCCGAGACACAG  
CAAGAAGGGAGGCGATTTCGTCGCAAGCCTGTTTCATCTCCGCACTGGCAACCTA  
CACATACAAAACCTTGAATTACCAACATCTCTCTAAAGTGGTGAACCAAGCCCT  
GGCTTACCTCCCCTATGCTACCAGCGCATTAATAATGTTTACCCCAACCGCGCT  
GGAGAGCGTGGTGATACTGAGCACCAGATATATAAAACATACCTCTCTATAAG  
GAAGGGGAAGAGTGATGGATTGCTGGGTACGGGGATAAGTGCAGCCATGGAA  
ATCCTGTACAAAACCCAGTATCGGTAGGTATATCTGTGATGTTGGGGGTAGG  
GGCAATCGCTGCGCACAAACGCTATTGAGTCCAGTGAACAGAAAAGGACCCTAC  
TTATGAAGGTGTTTGTAAAGAACTTCTTGGATCAGGCTGCAACAGATGAGCTGG  
TAAAAGAAAACCCAGAAAAAATTATAATGGCCTTATTTGAAGCAGTCCAGACAA  
TTGGTAACCCCTGAGACTAATATACCACCTGTATGAGATGAAAGCTTTACTACAAAGGTT  
GGGAGGCCAAGGAACCTATCTGAGAGGACAGCAGGCAGAACTTATTCACATTG  
ATAATGTTTGAAGCCTTCGAGTTATTAGGGATGGACTCACAAGGGGAAAATAAG  
GAACCTGTCCGGAATTACATTTTGGATTGATATACGGCCTACACAAGCAAAT  
CAACAGAGGGCTGAAGAAAATGGTACTGGGGTGGGCCCCTGCACCCTTTAGTT  
GTGACTGGACCCCTAGTGACGAGAGGATCAGATTGCCAACAGACAACTATTG  
AGGGTAGAAACCAGGTGCCCATGTGGCTATGAGATGAAAGCTTTCAAAAATGT  
AGGTGGCAAACCTTACCAAAGTGGAGGAGAGCGGGCCTTTCTATGTAGAAACA  
GACCTGGTAGGGGACCACTCACTACAGAGTCACCAAGTATTACGATGACAAC  
CTCAGAGAGATAAAACCACTAGCAAAAGTTGGAAGGACAGGTAGAGCACTACTA  
CAAAGGGGTACAGCAAAAATTGACTACAGTAAAGGAAAAATGCTCTTGCCA  
CTGACAAGTGGGAGGTGGAACATGGTGTGATAACCAAGGTTAGCTAAGAGATAT  
ACTGGGGTTCGGGTTCAATGGTGCATACTTAGGTGACGAGCCCAATCACCGTGC  
TCTAGTGGAGAGGGACTGTGCAACTATAACCAAAAACACAGTACAGTTTCTAAA  
AATGAAGAAGGGGTGTGCGTTACCTATGACCTGACCATCTCCAATCTGACCA  
GGCTCATCGAACTAGTACACAGGAACAATCTTGAAGAGAAGGAAATACCCACC  
GCTACGGTCACCAATGGCTAGCTTACACCTTCGTGAATGAAGACGTAGGGAC  
TATAAAACCACTAGTAGGAGAGAGAGTAATCCCCGACCCTGTAGTTGATATCAA  
TTTACAACCAGAGGTGCAAGTGGACACGTGAGAGGTTGGGATCACAATAATTG  
GAAGGGAAACCTGATGACAACGGGAGTGACACCTGTCTTGAAAAAGTAGA  
GCCTGACGCCAGCGACAACCAAACTCGGTGAAGATCGGGTTGGATGAGGGT  
AATTACCCAGGGCCTGGAATACAGACACATACTAACAGAAGAAATACACAA  
CAGGGATGCGAGGCCCTTCATCATGATCCTGGGCTCAAGGAATTCCATATCAA  
ATAGGGCAAAGACTGCTAGAAATATAAATCTGTACACAGGAAATGACCCAGG  
GAAATACGAGACTTGATGGCTGCAGGGCGCATGTTAGTAGTAGCACTGAGGGA  
TGTCGACCCTGAGCTGTCTGAAATGGTTCGATTTCAGGGGACTTTTTTAGATAG  
GGAGGCCCTGGAGGCTCTAAGTCTCGGGCAACCTAAACCGAAGCAGGTTACCA  
AGGAAGCTGTTAGGAATTTGATAGAACAGAAAAAAGATGTGGAGATCCCTAAC  
TGGTTTGCATCAGATGACCCAGTATTTCTGGAAGTGGCCTTAAAAAATGATAAG  
TACTACTTAGTAGGAGATGTTGGAGAGGTAAGATCAAGCTAAAGCACTTGG  
GGCCACGGATCAGACAAGAATTATAAAGGAGGTAGGCTCAAGGACGTATGCCA  
TGAAGCTATCTAGCTGGTTCCTCAAGGCATCAAACAAACAGATGAGTTAACTC

FIGURE 26-5

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CACTGTTTGAGGAATTGTTGCTACGGTGCCACCTGCAACTAAGAGCAATAAG  
GGGCACATGGCATCAGCTTACCAATTGGCACAGGGTAACTGGGAGCCCCCTCGG  
TTGCGGGGTGCACCTAGGTACAATACCAGCCAGAAGGGTGAAGATACACCCAT  
ATGAAGCTTACCTGAAGTTGAAAGATTTTCATAGAAGAAGAAGAGAAAACCT  
AGGGTTAAGGATACAGTAATAAGAGAGGCACAACAAATGGATACTTAAAAAAT  
AAGGTTTCAAGGAAACCTCAACACCAAGAAAATGCTCAACCCTGGGAAACTATC  
TGAACAGTTGGACAGGGAGGGGCGCAAGAGGAACATCTACAACCACCAGATT  
GGTACTATAATGTCAAGTGCAGGCATAAGGCTGGAGAAATTGCCAATAGTGAG  
GGCCCAAACCGACACCAAAACCTTTCATGAGGCAATAAGAGATAAGATAGACA  
AGAGTGAAAACCGGCAAAATCCAGAATTGCACAACAAATTGTTGGAGATTTTCC  
ACAGGATAGCCCAACCCACCCTGAAACACACACCTACGGTGAGGTGACGTGGGAG  
CAACTTGAGGCGGGGATAAATAGAAAGGGGGCAGCAGGCTTCTGGAGAAGA  
AGAACATCGGAGAAGTATTGGATTTCAGAAAAGCACCTGGTAGAACAATTGGTC  
AGGGATCTGAAGGCCGGGAGAAAGATAAAATATTATGAAACTGCAATACCAAA  
AAATGAGAAGAGAGATGTCAAGTGTGACTGGCAGGCAGGGGACCTGGTGGTT  
GAGAAGAGGCCAAGAGTTATCCAATACCCTGAAGCCAAGACAAGGCTAGCCAT  
CACTAAGGTCAATGTATAACTGGTGAAACAGCAGCCGTTGTGATTCCAGGAT  
ATGAAGGAAAGACCCCTTGTTCACATCTTTGATAAAGTGAGAAAGGAATTGG  
GACTCGTTCAATGAGCCAGTGGCCGTAAGTTTTGACACCAAAGCCTGGGACAC  
TCAAGTGACTAGTAAGGATCTGCAACTTATTGGAGAAATCCAGAAATATTACTA  
TAAGAAGGAGTGGCACAAGTTCATTGACACCATCACCGACCACATGACAGAAG  
TACCAGTTATAACAGCAGATGGTGAAGTATATATAAGAAATGGGCAGAGAGGG  
AGCGGCCAGCCAGACACAAGTGCTGGCAACAGCATGTTAAATGTCCTGACAA  
AGATTGACGCTTCTGCGAAAGCACAGGGGTACCGTACAAGAGTTTCAACAGGG  
TGGCAAGGATCCACGTCTGTGGGGATGATGGCTTCTTAATAACTGAAAAAGGG  
TTAGGGCTGAAATTTGCTAACAAAGGGATGCAGATTCTTCATGAAGCAGGCAA  
ACCTCAGAAGATAACGGAAGGGGAAAAGATGAAAGTTGCCTATAGATTGAGG  
ATATAGAGTTCTGTTCTCATACCCCAAGTCCCTGTTAGGTGGTCCGACAACACCA  
GTAGTCACATGGCCGGGAGAGACACCGCTGTGATACTATCAAAGATGGCAACA  
AGATTGGATTCAAGTGGAGAGAGGGGTACCAAGCATATGAAAAAGCGGTAG  
CCTTCAGTTTCTTGCTGATGTATTCTTGGAACCCGCTTGTAGGAGGATTTGCCT  
GTTGGTCTTTTCGCAACAGCCAGAGACAGACCCATCAAACATGCCACTTATTA  
TTACAAAGGTGATCCAATAGGGGCCTATAAAGATGTAATAGGTCCGAATCTAA  
GTGAAGTGAAGAGAACAGGCTTTGAGAAATTGGCAAATCTAAACCTAAGCCTG  
TCCACGTTGGGGATCTGGACTAAGCACACAAGCAAAAGAATAATTCAGGACTG  
TGTTGCCATTGGGAAAGAAGAGGGCAACTGGCTAGTTAACGCCGACAGGGCTGA  
TATCCAGCAAAACTGGCCACTTATACATACCTGATAAAGGCTTTACATTACAAG  
GAAAGCATTATGAGCAACTGCAGCTAAGAACAGAGACAAACCCGGTCATGGGG  
GTTGGGACTGAGAGATAAAGTTAGGTCCCATAGTCAATCTGCTGCTGAGAAG  
GTTGAAAATTCTGCTCATGACGGCCGTCGGCGTCAGCAGCTGAgacaaaatgtatatattgt  
aaataaattaatccatgtacatagtgatatataatagttgggaccgtccacctaagaagacgacagcccaacacgcacagctaaac  
agtagtcaagattatctacctaagataaactacattaatgcacacagcacttagctgtatgaggatacggccgacgtctatagttggac  
tagggaagacctctaacagcccc

FIGURE 26-6

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US99/08850

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A61K 39/29, 39/295; C12Q 1/70; C12N 7/01; C07H 21/02  
US CL :424/218.1, 228.1; 435/5, 235.1; 536/23.72

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/218.1, 228.1; 435/5, 235.1; 536/23.72

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS; Derwent/WEST; DIALOG

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P	FROLOV et al. cis-acting RNA elements required for replication of bovine viral diarrhea virus-hepatitis C virus 5' nontranslated region chimeras. RNA. November 1998, Vol. 4, pages 1418-1435, see entire document.	1-8, 10-21
Y,P	MALET et al. Yellow fever 5' noncoding region as a potential element to improve hepatitis C virus production through modification of translational control. Biochem. Biophys. Res. Commun. 18 December 1998, Vol. 253, No. 2, pages 257-264, see entire document.	1-8, 10-21

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
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Date of the actual completion of the international search  
19 JULY 1999

Date of mailing of the international search report  
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## INTERNATIONAL SEARCH REPORT

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PCT/US99/08850

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	LU et al. Poliovirus chimeras replicating under the translational control of genetic elements of hepatitis C virus reveal unusual properties of the internal ribosomal entry site of hepatitis C virus. Proc. Natl. Acad. Sci. USA. 20 February 1996, Vol. 93, No. 4, pages 1412-1417, see entire document.	1-8, 10-21
Y	VASSILEV et al. Authentic and chimeric full-length genomic cDNA clones of bovine viral diarrhea virus that yield infectious transcripts. J. Virol. January 1997, Vol. 71, No. 1, pages 471-478, see entire document.	1-8, 10-21
Y	VENUGOPAL et al. Towards a new generation of flavivirus vaccines. Vaccines. 1994, Vol. 12, No. 11, pages 966-975, see entire document.	1-8, 10-21

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US99/08850

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☒ Claims Nos.: 9  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:  
  
CLAIM 9 RECITES "SEQ ID NO:X" WHICH EXPRESSION IS NOT UNDERSTOOD.
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.  
☐ No protest accompanied the payment of additional search fees.

Form PCT/ISA/210 (continuation of first sheet(1))(July 1992)\*



## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<b>(51) International Patent Classification <sup>6</sup> :</b> <b>A61K 39/29, 39/295, C12Q 1/70, C12N 7/01, C07H 21/02</b>	<b>A1</b>	<b>(11) International Publication Number:</b> <b>WO 99/55366</b> <b>(43) International Publication Date:</b> 4 November 1999 (04.11.99)
<b>(21) International Application Number:</b> PCT/US99/08850 <b>(22) International Filing Date:</b> 23 April 1999 (23.04.99)  <b>(30) Priority Data:</b> 60/082,964                      24 April 1998 (24.04.98)                      US  <b>(71) Applicant (for all designated States except US):</b> WASHINGTON UNIVERSITY [US/US]; One Brookings Drive, St. Louis, MO 63130 (US).  <b>(72) Inventors; and</b> <b>(75) Inventors/Applicants (for US only):</b> RICE, Charles, M. [US/US]; 7316 Colgate Avenue, University City, MO 63130 (US). FROLOV, Ilya [RU/US]; 200 Tanglewood Drive, St. Louis, MO 63129 (US). McBRIDE, M., Scott [US/US]; 2807 Mickelson Pkwy. #205, Madison, WI 53711 (US). LEE, Young-min [KR/US]; 5530 Genesta Walk, St. Louis, MO 63123 (US). AGAPOV, Eugene, V. [RU/US]; 7515 Cromwell Drive, Apt. 2NE, St. Louis, MO 63105 (US). MYERS, Tina, M. [US/US]; 8141 Briarhaven Trail, Apt.102, St. Louis, MO 63123 (US).		<b>(74) Agents:</b> HOLLAND, Donald, R. et al.; Howell & Haferkamp, L.C., Suite 1400, 7733 Forsyth Boulevard, St. Louis, MO 63105-1817 (US).  <b>(81) Designated States:</b> AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).  <b>Published</b> <i>With international search report.          Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
<b>(54) Title:</b> CHIMERAS OF HEPATITIS C VIRUS AND BOVINE VIRAL DIARRHEA VIRUS  <b>(57) Abstract</b> <p>Disclosed is a polynucleotide comprising a chimeric viral RNA which contains: a 5' nontranslated region (5' NTR), an open reading frame (ORF) region, and a 3' nontranslated region (3' NTR) wherein at least one of said regions is chimeric. The chimeric region comprises a first nucleotide sequence from a pestivirus in operable linkage with a heterologous nucleotide sequence. The chimeric viral RNA is replication-competent. Preferably the pestivirus sequence is from a bovine viral diarrhea virus and the heterologous nucleotide sequence is from a hepatitis C virus. Also disclosed are a method for identifying compounds having antiviral activity against hepatitis C virus, a genetically-engineered chimeric RNA virus and a vaccine against bovine viral diarrhea virus.</p>		

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